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THE REGULATION OF RENAL ACTIVITY

I. Regulation of Urea Excretion by the Concentration of Urea in the Blood and in the Urine

T. ADDIS, G. D. BARNETT and A. E. SHEVKY

From the Medical Division of the Stanford University Medical School, San Francisco

Received for publication January 28, 1918

Renal activity expresses itself in the formation of urine from blood. But the urine is a complex product containing many substances which the kidney has separated from the blood. Each of these substances may call for a different type and degree of activity and each will require separate investigation before any comprehensive idea of the regulation of the action of the kidney as a whole can be attained. In the preliminary work on this general problem which is reported here, we have not attempted such an all inclusive study but have confined ourselves to the factors regulating the process of urea excretion. We felt that it would be advantageous to work at first with one particular substance and only to go on to others after we had obtained some experience in the difficulties which might arise and in the methods best adapted to meet them. We chose urea, partly because it is relatively easy to make accurate and numerous measurements of the urea content of the blood and urine, but mainly because we believed that the regulation of renal activity in the excretion of urea is simpler than that of other important substances dealt with by the kidney, such as water and chlorides. The reasons for this belief have been summarized elsewhere (1).

The excretion of urea, as of other urinary constituents is accomplished by the coördinated activity of "living" kidney cells, and from analogy with other biological phenomena it is to be expected that a large number of factors may directly or indirectly coöperate in determining the rate at which it is excreted and that there may be an intricate play of action and reaction among these factors. This probable complexity and our quite certain ignorance of even the main features of the process of urea excretion, indicate that the initial work must be of a simple qualitative nature. We must be satisfied, as a first step, with trying to find which of the many possible factors are actually operative and which of these are the more important.

With this end in view we have measured the rate of urea excretion in a large number of animals under certain fixed conditions. Having thus established an average rate and its range of variation, we have proceeded to observe the effect of experimental alterations in one after another of the possible factors, the conditions otherwise being kept as far as possible the same.

As originally planned, and up to a certain point carried out, it was intended that normal human individuals should be the subjects of study but problems presented themselves requiring procedures applicable only in animal experiments, and in the following attempts at their elucidation rabbits have been used. This change made it necessary to start the whole work again from the beginning, for the rabbit's average rate of urea excretion and its variability had to be found for the particular conditions chosen as our standard before it was possible to go on to estimate the degree of influence of any particular factor.

An *a priori* consideration of the subject would suggest that the factors determining the rate of urea excretion may be divided into two classes, those associated with the kidney itself and those associated with the blood and urine which form the immediate environment of the kidney cells. In general the first group will comprise limiting factors which are relatively constant—the quantity and quality of the structure of the renal secreting tissue—while the second group will contain factors which are unstable and fluctuating. If the influence on renal function of these two groups of factors could be separated in practice as well as in theory, if it were possible to measure the effect of the amount and quality of the secreting tissue of the kidney alone, this classification would be of fundamental importance for clinical medicine. It would allow of a distinction between those alterations in function which are of essential value in diagnosis and prognosis because they are permanent and determined by fixed anatomical peculiarities in renal structure, and those which are non-essential and transient because they have their origin in the ever changing environment of the kidney cells. Our ultimate object has been to draw such a distinction to at least such a degree as to render it of practical value. In attempting this we have first studied the factors associated with the immediate surroundings of the kidneys and have tried to eliminate the influence of the factors associated with kidney structure by working with rabbits of not very diverse weights and particularly by using the same animals repeatedly both under standard conditions and under conditions in which one or other of the factors considered was changed.

There is one variable factor, the concentration of urea in the blood, which would be first thought of as likely to be of importance in determining the rate of urea excretion. A comparison of rates measured over periods during which the blood urea concentration varies does in fact show that, as a general rule, the higher the level of the concentration of urea in the blood, the greater is the rate at which urea is excreted. Now we have not found it possible by any uniformity in external conditions to prevent the occurrence of considerable fluctuations in blood urea concentration even in the same animal. The inconstancy of this factor introduces an internal variable into our standard conditions which is beyond our power to control and whose effect is to produce such wide differences in rates measured under these standard conditions that the effect of any other factor experimentally introduced would have to be very marked indeed to be capable of demonstration. But the fact that this variable can be measured and taken into consideration in comparing rates, puts into our hands a means of obviating this difficulty. When we find that rates measured at the same blood urea concentration are nevertheless different, we may conclude that the difference is due to the intervention of other factors than blood urea concentration. Or when we find that by increasing the incidence of any particular factor we sensibly alter the average rate from that obtained at the same levels of blood concentration under our standard conditions, we may regard this alteration as an indication that the factor in question is operative, and we may take the nature and the degree of change which it induces as indicative of its mode of action and of its importance in comparison with other factors.

This first paper, accordingly, deals with the determination of the average rate of urea excretion under uniform external conditions for each level of blood urea concentration. This having been determined it becomes possible to estimate the influence of another variable factor in the environment of the kidney which can be accurately measured, i.e., the effect of variations in the concentration of urea in the urine. These questions have already been dealt with in our work on man, but apart from the fact that they form a necessary preliminary for further investigation on the rabbit, the observations here recorded extend the scope of the original study so far as the blood urea concentration is concerned, since the effect of a much wider range of variation in concentration could be determined than was possible under the restrictions required by care for the safety and comfort of human subjects.

METHODS

When the animals were not being used they were kept together in large cages in the animal room, except in springtime when it was found necessary to separate them in order to prevent them from killing each other in fights. They were all males. Once a day, about 10 a.m., they were given crushed oats, alfalfa and sometimes stale bread. Water was not restricted. When observations were to be made on any particular animal it was brought to the laboratory on the afternoon of the the previous day and placed in a small metabolism cage. No food and no water was given until the experiment, which began next day at 9 a.m., was ended. Our object was to exclude as far as possible such variations in kidney function as might be associated with differences in the food and water previously taken.

The conditions having thus been made uniform, at least to a certain extent, measures were taken to induce all degrees of variation, including the most extreme, in the concentration of urea both in the blood and in the urine. This was done by the administration of varying quantities of urea and of water.

The time-relationships which, quite unexpectedly, were found to be of considerable importance, were the same in all experiments. Starting in the morning about 9 a.m., the stomach tube was passed in all cases whether a urea solution or water or nothing at all was given. The animal was then placed on its back in a comfortable holder and the bladder emptied by catheter and washed with a known volume of water. It was then returned to a special cage built over a glass funnel so that if by chance any urine were passed between the periods of catheterization, it should not be lost.

Half an hour later a little more than 1 cc. of oxalated blood was obtained by puncturing the marginal ear vein after it had been dilated by warming over an electric light bulb. In the early part of the work, duplicate estimations on 5 cc. quantities were made; later duplicates on 1 cc. amounts, and during the last year with the successive improvements in the technique of aeration and titration which have been described by Barnett (2), we have usually carried out only one estimation on 1 cc. of blood.

One hour after the bladder had been emptied, the urine was collected by catheter, its volume measured and the bladder again washed out with a known volume of water, any excess returned, being added as a correction to the quantity already measured. The mixed urine and wash water was then diluted to an extent dependent on the quantity of urea expected with water containing sufficient H_2SO_4 to make it acid and so prevent decomposition of urea by urease containing organisms. Because they are easier to catheterize only male rabbits were used. Great care was taken to make sure that the bladder was completely emptied. The bladder is often so toneless that simple catheterization is not enough. The abdominal wall must be compressed, and the catheter alternately withdrawn, reintroduced and rotated. There is also a considerable degree of variation in the size of the urethra and the catheter selected must be large enough to prevent urine escaping by its side. A basin or funnel was used to catch urine which, in spite of all precautions, was sometimes passed in this way. In a considerable number of the experiments in which urea was not given, the washing of the bladder was omitted. We were inclined at first to think that the error in the calculation of the urea concentration of the urine which might be introduced by wash water left in the bladder, might be of greater importance than the increased accuracy which this procedure gives to the determination of the rate of urea excretion. In these experiments, however, the volumes of urine were large because of the diuretic effect of the urea, so that the error arising from leaving a little urine in the bladder was relatively small. In experiments in which the volumes of urine were small it was obvious that washing was imperative, and in all these cases it was done.

At the end of the second, third and fifth hours, the same process of catheterization was repeated, and at the middle of each of these periods blood was collected. For each experiment therefore four collections of urine were made, each with its corresponding collection of blood.

The urea estimations in both urine and blood were made with Marshall's urease method, using for the urine the titration method with the modifications we have already detailed, (3) and for the blood the aeration method with the slight changes which we described before, and latterly with the refinements introduced by Barnett (2).

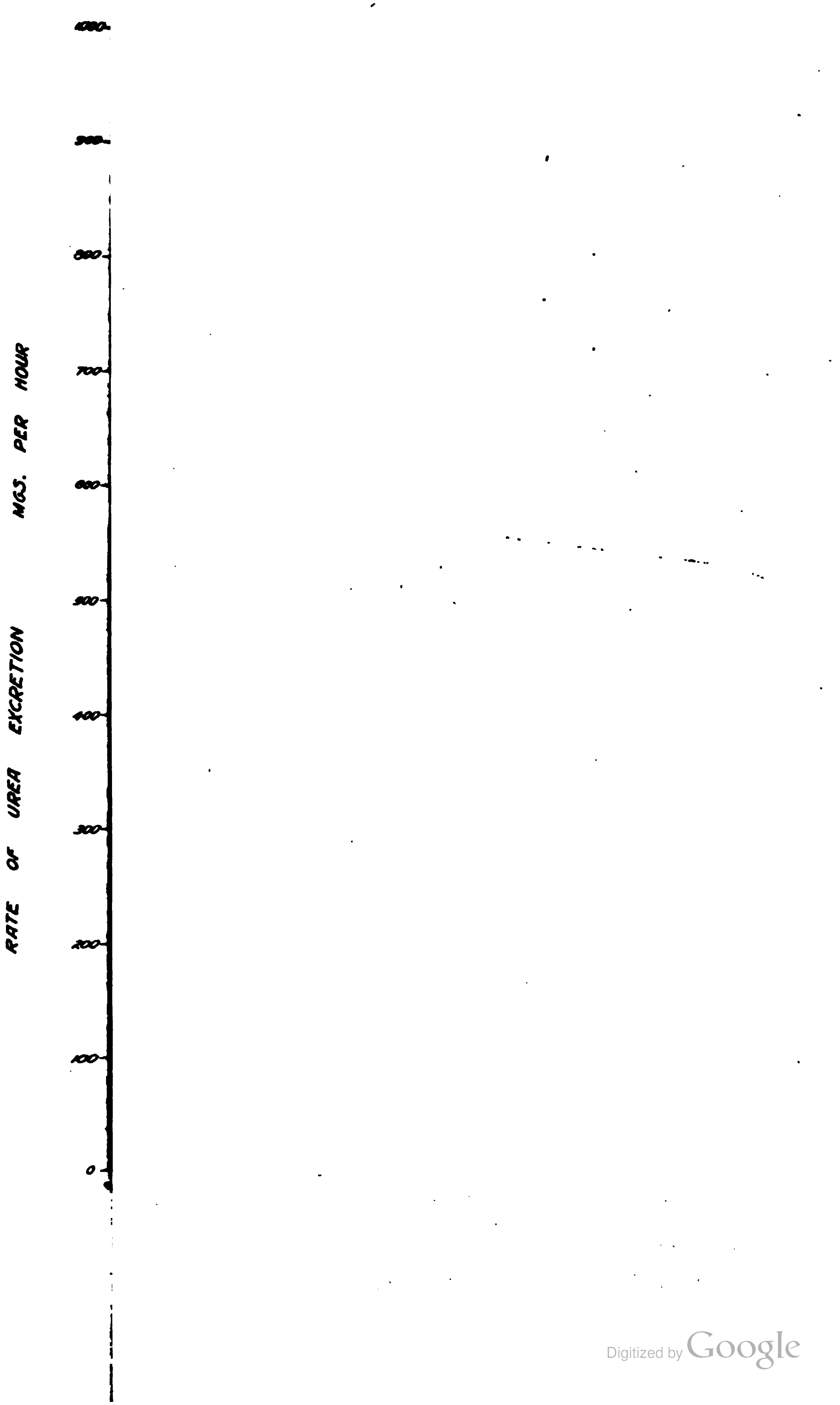
The rate of urea excretion, whether observed over a one or a two hour period, is in all cases given as the rate per hour in milligrams, the blood urea concentration as milligrams per 100 cc. of blood, and urine urea concentration as grams per 100 cc.

The data were collected in this manner because the effect of changes in blood or urine concentration on the rate of urea excretion should be capable of demonstration when the various rates, with the concentrations of urea found to exist during the periods over which they were measured, are compared. In the case of the data on the blood urea concentration this involves the assumption that the concentration measured at the middle of a period of urine collection represents the average concentration existing throughout the whole of that period. This of course is not necessarily the case, but since the periods of urine collection were never more than two hours, the error can seldom have been large and cannot invalidate merely qualitative deductions drawn from the general trend of a large number of observations.

It might also be questioned whether it is right to assume, as we in fact do, that the concentration of urea found in blood removed from an ear vein is the same as that in the blood supplied to the kidney, but since we were unable to find any significant difference in the urea concentration of blood obtained from the jugular vein, the carotid and the femoral artery, and the concentration in blood removed at the same time from the renal artery of the rabbit, we believe that this assumption is justified.

Regulation of urea excretion by the concentration of urea in the blood. In figure 1 each hourly rate of urea excretion is represented as a point on a chart in which the ordinate gives the magnitude of the rate and the abscissa the level of blood urea concentration observed at the time the rate was measured. In these circumstances if there is some relation between the two in the sense, for instance, that an increase in blood concentration is accompanied by an increase in rate, we should find that relation depicted as a rise in the ordinates from the left to the right of the chart. It will be seen that in a general sense there is evidence of such a relationship in the manner in which the points are grouped. The average rate is shown in the curve and demonstrates that, on an average, each increase in blood urea concentration is accompanied by an increase in the rate of urea excretion.

The very pronounced deviations of many of the rates from the curve of the average, make it almost unnecessary to point out that this curve cannot be used as a basis for any mathematical deductions as to the


RATE OF UREA EXCRETION MGS. PER HOUR
1000
900
800
700
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quantitative effect of changes in blood urea concentration on the rate of excretion. Before that could be done we should require the assurance either that all other factors had remained constant or that the factors which accelerated the rate had had their effect exactly neutralized by factors which depressed the rate. But mere uniformity in food and water supply and in the manipulations to which the animals were subjected, does not give us any right to suppose that either of these conditions was fulfilled in our experiments. These crude methods certainly did not, for instance, insure a constancy in the composition or in the amount of the blood supplied to the kidney. Therefore when we find, as we do, that by experimentally increasing the blood urea concentration we bring about an increase in the average rate of urea excretion, all we have accomplished is a demonstration that the level of blood urea concentration is a factor whose effect on the activity of the kidney in the excretion of urea is so marked that its influence can be traced in spite of the probable confusion and distortion introduced by the simultaneous inconstancy of other factors.

Regulation of urea excretion by the concentration of urea in the urine. Figure 1 shows that even at the same blood urea concentration there may be very wide differences in the rate of urea excretion. But though in these cases the blood urea concentration was constant, there was another possible factor, the urine urea concentration, which varied. It is possible that the inconstancy of this factor played an appreciable part in determining the observed differences in rates measured at the same blood concentration. From a strictly physical point of view, it may even be said to be probable. For we know that changes in the concentration of urea in the blood on one side of the kidney cell have an influence on the rate, and it would therefore seem likely that changes in the concentration of urea in the urine on the other side of the cell should also have some effect. The urea already taken from the blood into the cell should diffuse more rapidly into a urine of low urea concentration than into one in which the urea concentration was high, and vice-versa. If this were so we should find that those rates which are greater than the average, and thus lie above the curve in figure 1, are especially those in which the urinary concentration was low, and that those smaller than usual, and so falling below the curve, are those in which there was a high concentration of urea in the urine.

We have the data required for testing this hypothesis. If each rate is plotted on a chart in which the ordinate measures its distance above or below the curve in figure 1, and the abscissa the concentration of urea in the urine, we shall find, if it is correct, that the points will group themselves on either side of a line running downwards from the left to the right of the chart.

This has been done in figure 2. There is no evident grouping. The points are scattered more or less uniformly all over the chart so that the curve of the average deviation coincides roughly with the zero line of the ordinate scale. Therefore the particular hypothesis we put forward is incorrect, and further, no other hypothesis of the manner in which the urea concentration of the urine influences the rate of urea excretion is valid, since the chart indicates that there is no appreciable effect at all. The same conclusion necessarily follows in regard to the possibility of changes in the volume of urine being a factor whose influence is of sufficient importance to be capable of demonstration under the conditions of our experiments.

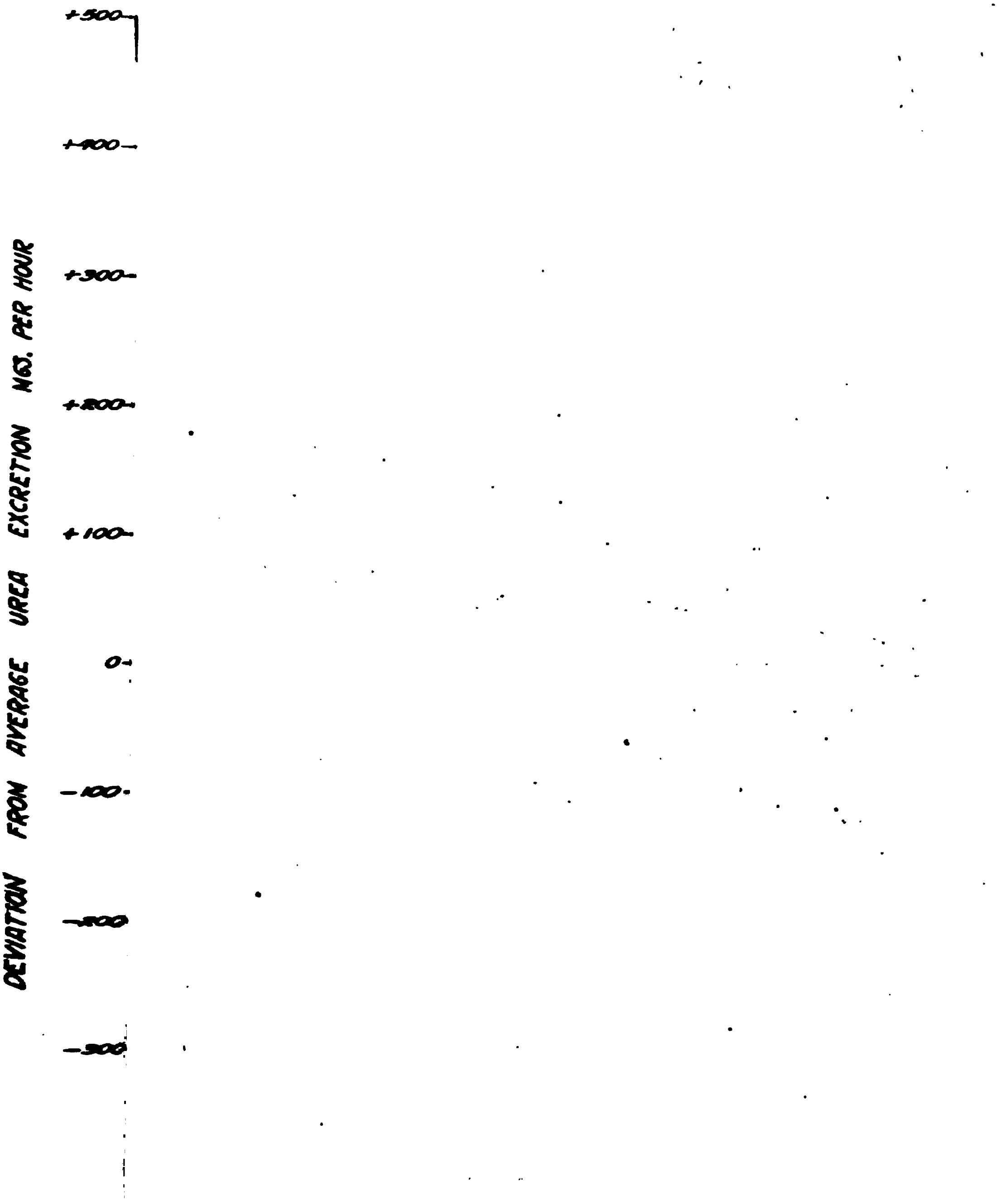
DISCUSSION

In a recent monograph Cushny says,

> The formation of the glomerular filtrate is due to a blind physical force, the absorption in the tubules is equally independent of any discrimination, for the fluid absorbed is always the same, whatever the needs of the organisms at the moment (4).

Those who hold such views will expect to find some relation between the urea concentration or volume of the urine and the rate of urea excretion. On the other hand the demonstration that the rate is not appreciably affected by these factors will not be surprising to those who are inclined to believe that the mechanical factors influencing the action of the kidney may be coördinated and overruled by a power which adapts them at every moment to the needs of the organism as a whole. Our experience seems to favor this latter view for it shows that in the excretion of urea the kidney is in some way freed from subjection to the physical forces originating in the composition of the urine within its tubules. That urine is no longer a part of the organism but is already outside it, and the adaptability of the kidney to the internal requirements of the body would be prejudiced if it were forced to conform its action to variations in its external and inert environment.

But if the work of the kidney is constantly adapted to meet the requirements of the organism as a whole, how are the wide differences in rates of urea excretion measured at the same blood urea concentration to be explained? So far as its urea excreting function is concerned one



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would have expected on this hypothesis to find the activity of the kidney directed toward the maintenance of an optimum concentration of urea within the body. This would involve a close and constant relation between the rate of excretion and the blood concentration so that whenever more urea entered the blood stream there would be a proportionate increase in excretion which would prevent any marked change in this particular component of the general equilibrium within the body. This is the mechanism with which we are familiar in the heat exchange of warm-blooded animals and in the excretion of such substances as carbon dioxide. But in the case of urea we find only a general average relation between the hourly rate of excretion and the blood concentration, and instead of a constancy in the concentration of urea in the blood we frequently find variations of over 100 per cent in normal individuals under physiological conditions (5). If we regard any particular blood urea concentration in figure 1, as representing the requirement of the body for the excretion of urea, then the degree of scattering of the rates at that level indicates how wide may be the difference between the amounts of work performed by the kidney in response to the same demand. However, apart from the possible fallacy of taking the blood urea concentration as the sole measure of the requirement of the body for urea excretion, it should be borne in mind that these differences are only found in rates observed over short periods of time. We made a special study of this point in man and found that the rate of excretion of pre-formed urea added to a constant diet was remarkably constant for times of twenty-four, twelve and even eight hours, and bore a close relation to the amount of excess urea in the body (6). It was only in rates measured for one-hour periods that marked discrepancies were found. Now if these short-lived irregularities are not be to regarded as instances of a failure in regulation, they might be interpreted as showing that temporary variations in blood urea concentration are a matter of indifference to the body. This possibility is supported by the fact that we have no knowledge that urea takes any part in the metabolic functions of the body, nor any evidence that such variations in blood urea concentration as are found interfere with any of the physical or chemical states essential for vital processes. It is therefore open to us to suppose that the forces regulating the activity of the kidney as a whole may, over short time periods at least, subordinate the excretion of urea to the more pressing requirements of the moment arising from the need for the retention or excretion of substances of greater physiological importance than urea. It will be noted that this conception implies that the functions of the kidney in the excretion of different substances are not entirely independent, so that a state of hyperactivity in the elimination of chloride, for instance, may be necessarily associated in some degree with increased work in the excretion of urea, in spite of the fact that no change may have occurred in the urea content of the tissues and blood. We have some experimental evidence, as yet incomplete, which seems to be in favor of this view, but this is a question which will require for its solution more direct evidence than any which we have as yet obtained. The immediately succeeding papers deal with the effect of factors of another variety and suggest another explanation for variations in rates measured at the same blood urea concentration.

CONCLUSIONS

1. In the rabbit the concentration of urea in the blood is an important factor in determining the rate of excretion of urea measured over short periods of time. Its effect, however, only becomes clearly apparent when the averages of many rates observed at different levels of blood urea concentration are compared, and at every level of blood concentration individual rates are found which are much higher or lower than the average.

2. Neither the concentration of urea in the urine nor the volume of urine are factors which appreciably influence the rate of excretion of urea under the conditions of our experiments.

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THE REGULATION OF RENAL ACTIVITY

II. Regulation of Urea Excretion by Anatomical Factors

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Although our immediate aim is the determination of the nature of the variable factors influencing urea excretion and although we accordingly adopted measures intended to exclude the influence of factors associated with the permanent structure of the kidney, it is necessary to consider to what extent we have failed in this endeavor and to attempt to define the degree to which anatomical peculiarities in the kidneys of the rabbits we used may have influenced our results. In this paper we have also considered some effects of anatomical factors on the rate of urea excretion in general.

It is conceivable that the rate of urea excretion is determined by only two factors, the blood urea concentration and the amount and quality of the urea secreting tissue of the kidneys. In that case differences in rates observed in a group of individuals in spite of constant blood concentration would arise from divergences in the structure of their kidneys. In that case also in the same individual the rate would be determined solely by the blood concentration. At the same concentration the rate would always be the same and at different concentrations we should find a constant relation between the rate of excretion and the level of blood urea concentration. It is, of course, not difficult to show that this conception is too narrow and rigid to account, even approximately, for the complexities of the process of urea excretion. In the same individual, for instance, rates may vary widely at the same blood concentration. We reproduce data from single rabbits in illustration of this point.

In these individual cases we see that the same kidneys manifest differences in rates measured at the same blood concentration; differences which are not appreciably smaller than those shown between the rates from the group of many different kidneys which are charted in figure 1 of the preceding paper. It is therefore evident that such anatomical divergences as may have existed between the kidneys of this group had only a relatively insignificant influence in the production of these differences. The small and constant differences which minor structural variations induce are obscured by the large and variable differences arising from the operation of unknown factors.

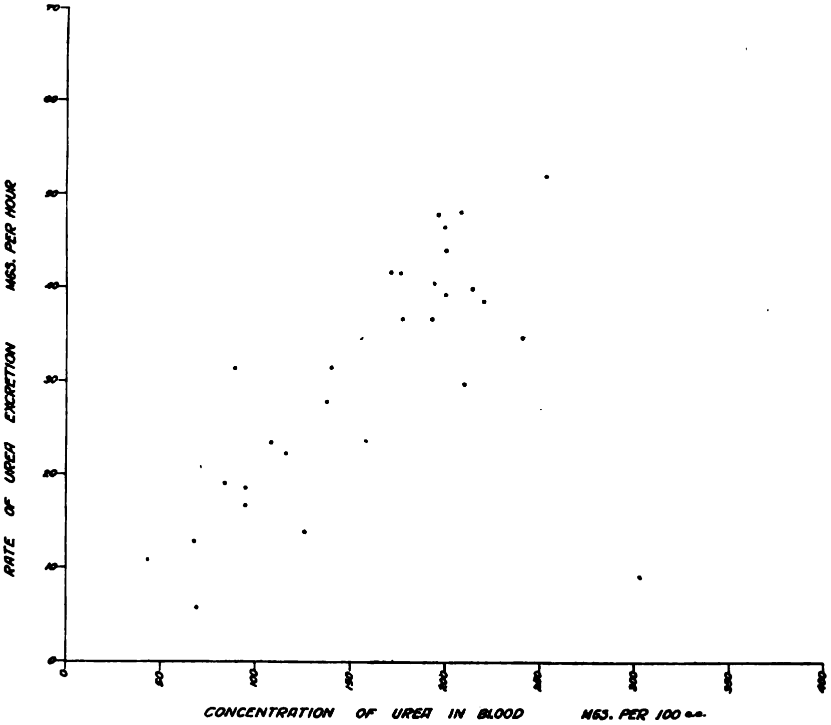

Fig. 1. Observations on a single rabbit showing that in the same individual the rate of urea excretion may vary widely though measured at the same or approximately the same blood urea concentration.

We have not made any detailed and systematic study of the degree of anatomical divergence which is required to produce a difference in function so marked that it will not be obscured by variable factors. From the practical point of view that is a question of great importance, but it would be best to undertake it when more is known as to the nature of these unknown factors and after means are devised under which their action is controlled or rendered less variable. From the few observations we have made under the conditions of our present experiments, it would appear that within the same species it is only when differences between amounts of kidney tissue transcend the range of normal adult variability, that a corresponding difference in function becomes clearly apparent.

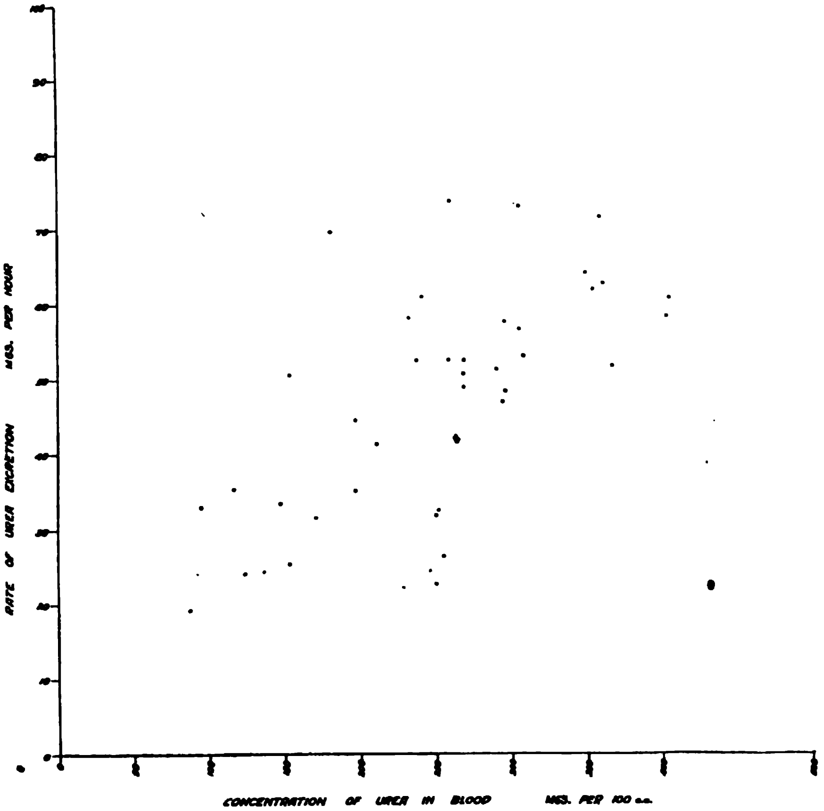

Fig. 2. Observations on a single rabbit showing that in the same individual rates may vary widely though measured at the same or approximately the same blood urea concentration.

In two unusually large rabbits, each weighing about 3000 grams, the average of twelve hourly rates of urea excretion was 421 mgms., the blood concentration averaging 197 mgms. per 100 cc. In four very small rabbits ranging from 350 to 515 grams, the average of sixteen determinations carried out under the same conditions was only 130 mgms., although the urea concentration was 243 mgms. per 100 cc. Therefore marked differences in body weight, and so presumably in kidney weight, between animals of the same species, are sufficient to lead to quite pronounced differences in rates of urea excretion.

We used only medium sized animals in all other experiments, but it was not feasible to adopt a very rigid standard and it is probable that some part of the variability in rates measured at high blood concentrations arose from differences in the quantity of renal tissue in our animals.

We also could not be sure that the qualitative anatomical changes produced in the kidney by either general or local disease were not important factors in certain cases. Our rabbits unfortunately were not always in good health. A considerable number died from natural causes. In these, coccidiosis was usually the only gross lesion discovered. There was also a condition, frequently only temporary, characterized by loss of weight and by a rise in blood urea concentration, usually most pronounced in those animals in which the greatest emaciation had occurred. But there was not by any means always an appreciable defect in the urea excreting capacity of the kidney in this condition. Indeed, we sometimes saw kidney function continue undisturbed almost up to the very moment of death. On the other hand, we occasionally came across animals in which renal activity was at a level much lower than the average, although they presented no signs or symptoms of disease. Even post mortem examination of the kidneys did not give any sure guidance in determining the normality or abnormality of our animals. This was because we used the rabbits in repeated experiments and only examined the kidneys when they died from natural causes. The acute diffuse degenerative renal lesions which were found in some of these cases could rarely be associated with functional changes, since the time at which they originated could not be fixed. There was also, of course, the usual high percentage of cases of so-called spontaneous nephritis, a focal interstitial lesion which did not appear to have any appreciable effect on function. Chemical and microscopical examination of the urine would perhaps have been the best way to exclude animals with diseased kidneys, but as is well known, albuminuria is almost constant in caged rabbits, and from the nature of the urine casts are often difficult to find.

In the end we decided that, since we had no very reliable criterion, it would be better to make no attempt at selection at all. The only observations we have rejected are those made on animals which died during the course of the experiment or very shortly afterwards. In preliminary work of this sort in which a considerable mass of data has been collected, the inclusion of all observations is perhaps the most satisfactory method. For though we thereby lose precision and detail in our deductions, we may at least feel sure that any general conclusions which may be reached are unbiassed by a selection, necessarily more or less arbitrary, from which it would have been hard to exclude the personal factor.

We have confined discussion so far to the influence of anatomical factors as possible causes of differences between rates of urea excretion observed in animals of approximately the same size and measured at the same blood urea concentration, that is to say as possible explanations of the high degree of scattering shown in figure 1 of the first paper. It is only from this aspect that any influence they may have is of moment so far as our present investigation is concerned. We have shown that from this point of view their effect is small and uncertain.

TABLE 1

Average rates of urea excretion in the rabbit and in man at different levels of blood urea concentration

UREA IN 100 CC. OF BLOOD	UREA IN ONE HOUR'S URINE	
	Rabbit	Man
mgms.	*mgms.*	*mgms.*
40	32	1100
50	45	1550
60	59	2100
70	79	2600
80	102	3150
90	125	3685

But from a wider standpoint the anatomical factor of kidney size is of primary importance in regulating the rate of urea excretion. Other factors may regulate the activity of the kidney so that a quite wide range of rates is found at any given blood urea concentration, but the value of the average rate round which these variations occur is determined by the size of the kidney. This becomes apparent on comparing the average rates yielded by kidneys of widely different sizes, as for instance the kidney of man and of the rabbit. In the above table the contrast is carried only as far as a blood concentration of 90 mgms., since no average based on a sufficient number of observations was obtained from man beyond that point.

It is here shown that the average rate in man is rather more than thirty times greater than in the rabbit. Approximately a man weighs thirty times more than a medium sized rabbit. From the relation which exists between body weight and kidney weight there is presumably about thirty times more renal tissue in man than in the rabbit. The order of magnitude of the average rate of urea excretion appears therefore to be a function of the size of the kidney.

The form of the curve of the rate of urea excretion is different from the similar curve in man. The form of the curve of the ratio between the urea content of the urine and of the blood is also necessarily different. This ratio is an expression we have found convenient, especially for statistical purposes. It is obtained by dividing the urea content of one hour's urine by the urea content of 100 cc. of blood. When the rates charted in figure 1 of the first paper are plotted as ratios, the curve of the average ratio is seen to rise to a maximum and then to fall (see fig. 3). The similar curve in man shows no such declension.

But in man the curves were only carried to a concentration of 100 mgms. per 100 cc. while in the rabbit they are carried as far as 400 mgms. per 100 cc. If we had forced the blood urea concentration as high in man as in the rabbit the analogous curves might have followed the same course. Within the concentrations at which they can be compared, there is no essential difference.

The gradual rise of the ratio curve up to a certain level of blood concentration and its subsequent decline at still higher levels resembles the type of curve obtained when the work output of a muscle is charted under successively increasing loads. The rise in the ratio might then be regarded as the effect of strain on the activity of the kidney and its fall as the effect of over-strain.

But the mode of energy transmission is so radically different in muscle as compared with kidney tissue, that this analogy may be misleading. It is also necessary to be cautious in drawing deductions from the form of curves constructed from such heterogeneous data as ours. It seemed possible, for instance, that those observations made when the blood urea concentration was exceptionally high might have been drawn in the main from animals whose kidneys were diseased. The fall in the curve would be much more convincing if it were shown to occur in animals whose reaction to lower urea concentrations had been shown to be normal.

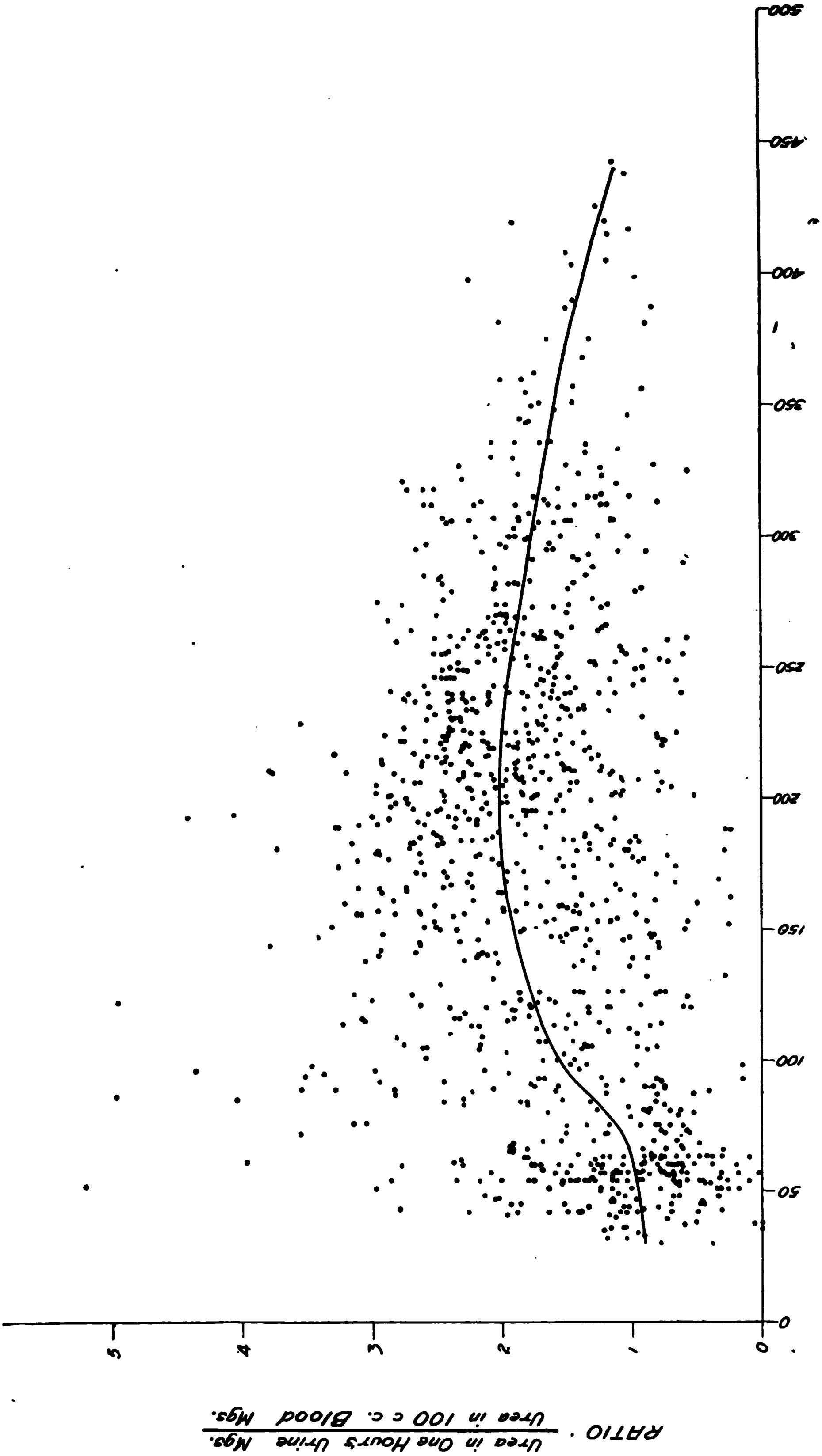

Fig. 3. The effect of changes in the concentration of urea in the blood on the ratio between the urea content of the urine and the blood.

Unfortunately we have few animals on which observations were made at several widely differing blood concentrations, but there is one group of seven rabbits on which experiments after no urea, 5 grams and 10 grams of urea were carried out. The averages are given in table 2.

These figures lend no support to the supposition that the fall of the ratio curve in figure 3 at blood concentrations over 225 mgms. can be due entirely to the inclusion of animals with diseased kidneys. For this group, whose kidneys reacted normally at low and medium levels, shows a quite definite and constant decrease in the ratio at concentrations over 300 mgms. as compared with those measured at about 200 mgms. We may therefore conclude that the decline of the curve of the ratio in the group chart represents, qualitatively at least, the true reaction of the normal kidney to very pronounced and sudden increases in blood urea concentration, though quantitatively it may be somewhat exaggerated by occasional instances of decreased function due to pathological causes.

TABLE 2

A comparison of average rates of urea excretion and of average ratios from the same group of animals as measured at low, medium and high blood urea concentrations

AMOUNT OF UREA ADMINISTERED	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in one hour's urine	Urea in 100 cc. blood	Ratio	Urea in one hour's urine	Urea in 100 cc. blood.	Ratio	Urea in one hour's urine	Urea in 100 cc. blood	Ratio	Urea in one hour's urine	Urea in 100 cc. blood	Ratio
grams	*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*	
0	48	64	0.62	81	71	1.10	79	70	1.05	104	73	1.53
5	134	145	0.99	333	190	1.84	431	207	2.18	409	219	1.93
10	261	210	0.96	510	307	1.68	671	375	1.69	668	382	1.83

Though the hypothesis of over-strain is thus supported there is no reason to suppose that this decrease in the ratio between urea excreted and urea still to be excreted is the result of a process similar to the "fatigue" of muscle. There is no evidence that the products of the metabolic activities of the kidney accumulate so as to hamper the work of the secreting cells. And there is no relation between the time during which a kidney has been under strain and the degree of decrease in the ratio. In the above example, for instance, the decrease after 10 as compared with 5 grams of urea is more marked in the second and third periods than in the last. And in experiments on rabbits in which the blood urea concentration was kept for days and even weeks at an almost continuously high level by repeated doses of urea, we never found any change in function which could be interpreted as the result of fatigue.

There is, however, a factor which would account for a decrease in ratios at very high blood urea concentrations. When the blood concentration is forced higher and higher by the administration of increasing quantities of urea, theoretically at least, a concentration will at last be produced at which the rate of urea excretion will have reached the maximum to which the quantity of renal tissue present is capable of attaining. There the curve of the rate must cease to rise and must thereafter move horizontally. There, also, the curve of the ratio must commence to decline in proportion to every further increase in blood urea concentration. That concentration represents the amount of work in urea excretion which taxes all the urea secreting tissue of the kidney to its utmost capacity.

We believe that the flattening of the rate curve and the fall in the ratio curve at concentrations above 225 mgms. per 100 cc. do, in fact, represent the effect of the limiting factor of kidney size. That in our curves the change in direction should not be sharply defined is to be expected, when it is remembered that they are averages compiled from data on a considerable number of animals, each with its own individual renal capacity.

The only reason for doubt lies in the possibility that the diminution in the relative activity of the kidney represented by the change in the direction of the curves might arise from secondary effects and not as a direct result of an overburdening of the kidney tissue by the urea itself. For though urea is perhaps of all substances the one to which the body is most indifferent, yet when such enormous quantities are given as are required to raise the blood urea concentration to over 225 mgms. per 100 cc. in subjects whose kidneys are normal, we are doing more than increase the work the kidney is called on to perform in the excretion of urea. These great concentrations more or less suddenly induced, must tend to disturb the balance of molecular concentration in the tissues. It has been shown also that the intravenous injection of 80 per cent urea solutions leads, in rabbits, to an alteration in the haemoglobin percentage of the blood, presumably due to an increase in its water content (1). And in man symptoms such as headache and inability for physical or mental exertion are induced by the administration by mouth of 100 to 120 grams of urea, amounts which, though they in no case raised the blood urea concentration much over 200 mgms. per 100 cc., were yet followed by a reduction in the haemoglobin percentage (2). Further, rabbits are susceptible to poisoning by ammonia derived from the bacterial decomposition of unabsorbed urea reaching the large intestine (3). A number of our animals died from this cause after 5 grams of urea (4) and apparently the greater the quantity of urea given the more frequently does ammonia poisoning occur. In the above group of seven, only two escaped after doses of 15 grams. Instances of non-fatal ammonia poisoning were occasionally seen, so that it is not unlikely there were a number of minor undetected cases.

All these secondary effects of large doses of urea may have combined to embarrass the work of the kidney, and if we had not seen so many instances of urea excretion remaining undisturbed under procedures involving much more serious general disturbances than the hypothetical ones we have mentioned, we should have been inclined to attach more importance than we do to this possible explanation.

Though the change in direction of the rate and ratio curves is due to the limitation on function imposed by the structural capacity of the kidneys yet under physiological conditions the reaction of the kidney to its environment is in no way influenced by this ultimate anatomical limitation. This is only a potential factor in the regulation of renal activity, operative in extreme cases of reduction of kidney size by disease, or in the entirely artificial condition arising from the ingestion of large quantities of urea. The normal kidney has a possible range of action much greater than that which it actually covers. For if the average blood urea concentration of the rabbit be taken as 30 mgms. per 100 cc., the fact that the ratio continues to increase up to a level of 225 mgms. indicates that the capacity of the kidney is about seven times greater than that which is just sufficient. In anatomical terms, it shows that there is seven times more renal tissue than is ordinarily called into full action.

CONCLUSIONS

1. The size of the kidney determines the order of magnitude of the average rate of urea excretion at all blood urea concentrations.

2. In medium sized rabbits such differences as presumably exist between the amounts of renal tissue they possess are too small to account for any but a small part of the marked differences which occur between rates of urea excretion measured at the same blood urea concentration.

3. There is no marked increase in the average rate of urea excretion in rabbits when the blood urea concentration rises higher than 225 mgms. per 100 cc. This is interpreted as indicating that about that level of blood urea concentration, the activity of the kidney becomes limited by the factor of kidney size.

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THE REGULATION OF RENAL ACTIVITY

III. Regulation of Urea Excretion by Unknown Factors

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The excretion of urea is regulated not only by the blood urea concentration and by the amount of active secreting tissue in the kidney, but also by other and unknown factors. The operation of these factors is demonstrated by the occurrence in the same animal of differences between rates measured at the same blood concentration, and their scope of action is indicated by the extent of these differences. In this paper an analysis of some general features of these differences has been made in the hope that some information might be obtained in regard to the mode of action of the factors through whose intervention they arise, and especially in order to obtain a foundation for deductions as to the method best adapted for a more particular investigation of their nature.

The effect of the unknown factors varies progressively in consecutive observations. It will be remembered that in each experiment four consecutive collections of urine and of blood were made. When no urea was given, the blood urea concentration remained at about the same level throughout each of these four periods, and one might have confidently expected that the rate of urea excretion, though exhibiting irregular fluctuations, should on an average also have remained at approximately the same level since no food had been taken for seventeen hours and there was no apparent reason why the formation and elimination of urea should not have proceeded at a more or less even pace.

We were therefore surprised to find that the rate of urea excretion showed a pronounced and progressive increase in each successive period of the experiment. In table 1 we give the averages in each period of the rate of urea excretion and blood urea concentration and of the ratio between the urea content of the urine and of the blood. These were obtained from forty-three experiments on a group of thirty-four rabbits which received neither water nor urea. These averages are given in table 1 charted in figure 1 and the details for each animal are given in table 2 at the end of the paper.

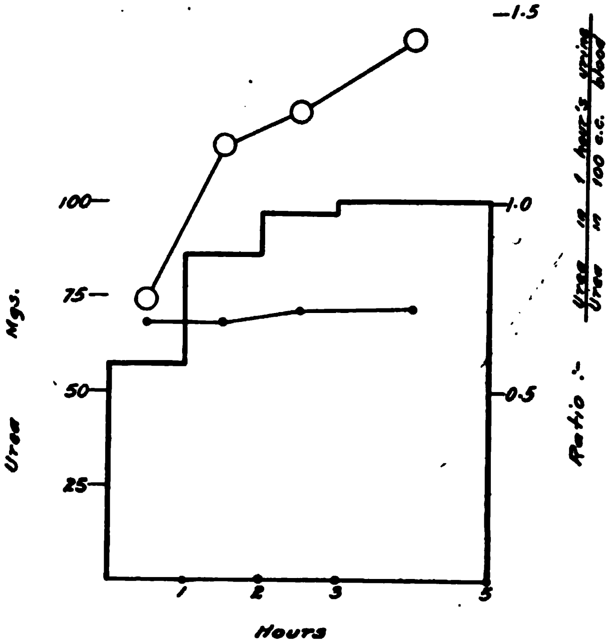

Fig. 1. Showing the increase in the rate of urea excretion in each successive observation, in spite of an approximate constancy in blood urea concentration.

The hourly rate of urea excretions represented by the blocked areas. The blood urea concentration is shown by dots joined by lines. The values for both the rate and the blood concentration are given by the scale on the ordinate at the left of the chart.

The ratio between the urea in one hour's urine and the urea in 100 cc. of blood is given as circles joined by lines. The value is shown by the ordinate at the right of the chart.

TABLE 1

Rate of urea excretion in consecutive observations during which the blood urea concentration remained constant. Averages of 43 experiments on a group of 34 rabbits

PERIOD	UREA IN ONE HOUR'S URINE	UREA IN 100 CC. BLOOD	*RATIO:* $\frac{\text{UREA IN ONE HOUR'S URINE}}{\text{UREA IN 100 CC. BLOOD}}$
	mgms.	*mgms.*	
First hour	57	68	0.74
Second hour	86	68	1.15
Third hour	97	71	1.24
Fourth and fifth hours	100	72	1.43

The blood urea concentration remains practically constant, varying only between 68 and 72 mgms. and yet the hourly rate of excretion increases from 57 mgms. in the first period to 86 mgms. in the second, to 97 in the third and to 100 mgms. in the last. This means that in some way an increase in the urea excreting activity of the kidney had gradually taken place for at its highest point, where 100 mgms. are excreted per hour or 2.4 grams per 24 hours, we have a rate which is considerably greater than that shown by medium sized rabbits for the first 24 hours of abstinence from food and water, when they are left undisturbed and are not subjected to manipulation.

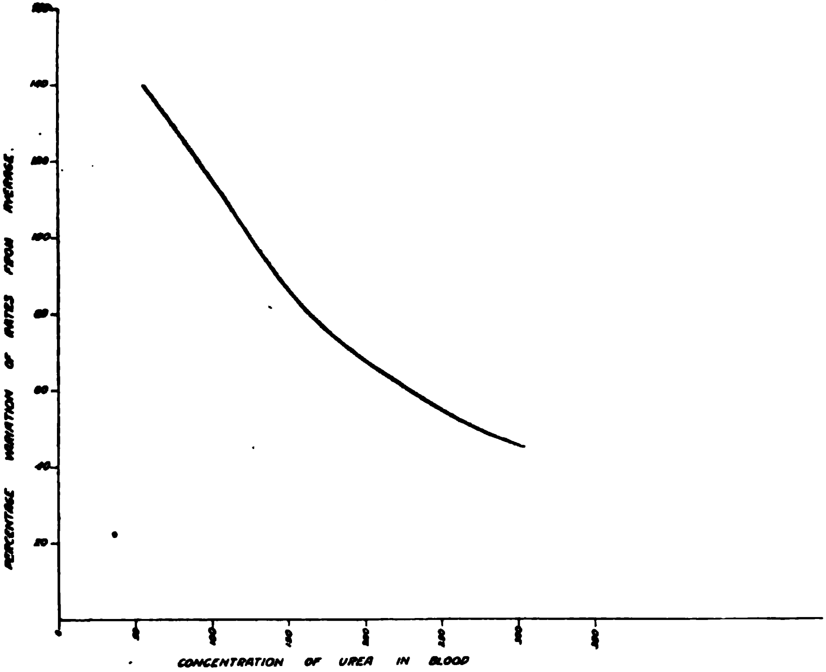

Fig. 2. Showing the decrease in the variability of the rate of urea excretion as the concentration of urea in the blood increases. Variability is measured by the percentage standard deviation from the average rate of urea excretion.

This progressive rise can be attributed neither to anatomical factors nor to changes in blood urea concentration. It is an example of the effect of unknown factors which so regulate the action of the kidney as to lead to an increasing efficiency in the excretion of urea with the passage of time from the commencement of the experiment.

Conditions which influence the variability in urea excretion produced by unknown factors. A knowledge of the conditions under which the operation of these unknown factors is most clearly manifested is of importance in the selection of the experimental conditions best adapted for their study; for an experimental intensification of a factor suspected of belonging to this group may perhaps only yield a significant deviation from the usual mode of action of the kidney if the control standard has been established under conditions in which the kidney is most sensitive to the influence of the factor in question.

Undoubtedly the most important of these conditions is that the time over which kidney function is measured should be short. The unknown factors produce only evanescent fluctuations in kidney activity which rise and fall and quickly tend to counterbalance one another (1). Their action must therefore necessarily be investigated over short time intervals such as we have adopted.

The level of blood urea concentration is another condition which has a considerable influence. The absolute variability at each level of blood concentration is shown in the degree of deviation of the points above and below the curves of the average in the rate and ratio charts. But the true measure of variability is, of course, relative and not absolute. In figure 2 the curve of the percentage standard deviation from the average rate is given.

It will be noted that the variability is greatest when the blood urea concentration is low, and decreases the higher it rises.

The time relationships of consecutive observations also influence the variability, for during the first hour the variability is higher than in subsequent periods. Thus in thirty-five experiments on a group of twenty-seven rabbits, whose blood urea concentration remained at a constant level, the standard deviation of the ratio was 43 per cent of the arithmetical mean in the first period, and fell to 34 per cent, 35 per cent and 36 per cent respectively for the three remaining periods.

DISCUSSION

This analysis of the data given in the first paper of this series allows us to form some conclusion as to the methods most likely to prove effective in an inquiry into the nature of the factors we have grouped under the term unknown.

In the first place, the great variability of rates or of ratios measured at constant blood concentration under our standard conditions makes it plain that statistical methods must be applied before weight can be attached to deviations found under experimental conditions. It will manifestly not be enough to compare single experiments. The averages of groups must be the unit of comparison and the degree to which chance might account for any observed difference between averages must be calculated and taken into account.

We have shown that it is highly probable that in certain cases anatomical factors played a part in the production of such differences between rates or ratios in different animals as were not due to variation in blood urea concentration. Since we wish to eliminate all factors associated with structural peculiarities, we must obtain our average results on a group of animals under standard conditions and then repeat the work on the same group under, as far as possible, the same conditions except for the introduction of the factor whose effect we wish to test.

The remarkable increase in rates and ratios over consecutive hours of bleeding and catheterization indicates that in watching for the effect of any experimental factor, we must not be satisfied with the average rate over the whole five hours of observation, but must also attach importance to any statistically significant deviation from the progressive increase in successive hourly rates and ratios, which we have found to be characteristic of the mode of regulation under the conditions adopted as our standard.

Finally, we may expect to find the kidney more susceptible to the effect of the factors under investigation over short time periods when the blood urea concentration is low and particularly during the first period of observation.

CONCLUSIONS

1. There is a progressive increase in the rate of urea excretion in consecutive observations on rabbits subjected to catheterization and bleeding, so that at the last the rate is nearly twice as great as at the commencement of the experiment and exceeds the rate yielded by the rabbit under the same conditions except for the absence of handling. This increase in the activity of the kidney occurs in spite of the absence of any change in blood urea concentration.

2. The variability of rates or ratios measured at the same blood urea concentration is greater the shorter the time of observation, decreases as the blood urea concentration increases, and in a series of consecutive observations is most pronounced during the first.

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TABLE 2

Control experiments. No urea

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood	Urea in 1 hour's urine	Urea in 100 cc. blood	Urea in 1 hour's urine / Urea in 100 cc. blood
	mgms.	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*		*mgms.*	*mgms.*	
59*	27	50	0.53	38	52	0.75	50	55	0.90	60	59	1.03
65*	112	126	0.91	167	125	1.33	174	127	1.39	197	137	1.45
66*	44	58	0.76	64	56	1.16	59	56	1.06	81	54	1.47
67*	62	55	1.14	85	57	1.50	97	56	1.75	98	57	1.75
68*	34	40	0.80	40	38	1.10	29	44	0.70	66	50	1.31
69*	56	59	1.01	86	61	1.30	102	63	1.51	107	63	1.65
70*	18	43	0.41	55	45	1.22	87	45	1.47	95	45	2.14
71	27	54	0.49	44	57	0.76	52	57	0.92	76	60	1.26
72*	43	65	0.65	61	66	0.88	67	65	1.03	78	66	1.65
73	37	54	0.69	64	57	1.12	83	54	1.53	127	54	2.35
80	67	90	0.75	125	65	1.94	144	62	2.32	167	60	2.77
82	215	150	1.43	351	50	2.34	321	164	2.02	lost	lost	lost
83	177	126	1.40	199	136	1.45	229	147	1.55	221	148	1.50
85	143	120	1.34	209	117	1.79	252	120	2.11	216	121	1.78
86	150	156	0.97	177	153	1.16	177	141	1.26	203	150	1.35
87	155	146	1.07	191	138	1.39	302	141	2.14	315	138	2.28
88	18	55	0.33	41	56	0.74	41	56	0.74	71	57	1.24
89	89	60	1.47	119	66	1.80	126	66	1.91	154	54	2.85
90	32	45	0.70	45	47	0.97	61	50	1.24	72	54	1.33
91	38	56	0.68	79	54	1.47	63	57	1.10	91	57	1.59
92	37	48	0.64	73	54	1.36	62	54	0.77	94	60	1.56
93*	27	39	0.66	39	38	1.05	39	45	1.15	59	55	1.25
94	23	53	0.44	21	53	0.40	40	60	0.68	13	60	0.20
95	81	90	0.90	154	93	1.67	168	112	1.50	62	160	0.50
96	35	44	0.80	80	41	1.96	83	45	1.85	62	54	1.15
97	29	42	0.70	45	44	1.02	68	45	1.52	87	45	1.93
98	0	38	0.00	47	44	1.06	52	46	1.14	98	48	2.13
99	54	49	1.10	54	47	1.15	60	54	1.11	63	51	1.24
100	0	36	0.00	2	38	0.06	9	51	0.18	16	48	0.34
101	11	57	0.19	17	51	0.32	18	57	0.32	5	54	0.10
102	7	63	0.10	2	57	0.03	25	54	0.46	31	60	0.53
103	43	42	1.04	69	42	1.64	58	44	1.33	83	51	1.64
104	33	48	0.69	40	51	0.79	46	58	0.79	54	60	0.91
105	13	51	0.26	36	57	0.63	46	69	0.66	69	75	0.93
Averages....	57	68	0.74	86	68	1.15	97	71	1.24	100	72	1.43

* The asterisk indicates that in the case of the animals whose number is so marked the results given are the average of two experiments.

THE SALIVARY FACTOR IN RELATION TO DENTAL CARIES

PERCY R. HOWE AND MILDRED R. KENISTON

From the Laboratory of the Forsyth Dental Infirmary for Children, Boston

Received for publication February 4, 1918

The power of the saliva to maintain a protective function over the buccal cavity and teeth has long been a subject for investigation, but little has been proven. That one of the functions of this fluid is to protect and benefit in some way the tissues which it continually bathes is sine qua non and the study of the saliva is an important phase in the understanding of the etiology of dental caries.

Miller (1) in 1890 suggested that in the saliva

> the great amount of carbonate of the alkalies is specially noteworthy; it imparts a strong alkaline reaction to the animal saliva, and is well calculated to neutralize such acids as may be found in the animal mouth, thus tending to prevent the appearance of caries.

Acids were considered the arch enemies of the teeth and it was a natural trend of research workers in dentistry to look for something to counteract any acidity in the mouth.

Marshall (2), however, in 1915 stated that the saliva is amphoteric and therefore for it to be a factor in protecting the teeth it must neutralize either acid or alkaline substances as taken into the mouth, and that it is the degree of this power to maintain a neutrality in the mouth which is indicative of susceptibility to caries.

He promulgated a theory of a "salivary factor" as an index of immunity from caries, which Gies and coworkers (3) could not substantiate by any of their work.

This report deals with a like investigation to find out what relation there may be between the salivary factor and the incidence to dental caries.

Marshall's procedure was followed using 10 cc. of the sample and titrating immediately after collection with N/200 NaOH and N/200 cc. HCl. Phenolphthalein and paranitrophenol were used as indicators. Paraffin was chewed as a stimulus for the activated saliva.

The saliva in all cases was diluted with freshly distilled water. This was done because in some instances the saliva was so dark colored that it would have been impossible to detect any color change, and it was always difficult to obtain more than 10 cc. of resting saliva from any patient.

All of the subjects were of about the same age, ten to fifteen years. These limits were set, as it is between these years that incidence to decay is most active, and 1500 children visiting the Infirmary every month for treatment made careful selection of cases comparatively easy. Those children with occasional fillings were discarded, as were also those with only one or two cavities. Absolute immunity and excessive decay only were chosen for the work to be done.

Note was taken of sex, general appearance, nationality, cleanliness of mouth, the time after a meal at which the saliva was collected and the character of the saliva. The resulting figures for each case were referred then to these things.

The patient was left alone during collection of the sample so that there would be no embarrassment nor any outside psychical excitation. As near normal conditions as possible were sought.

The following table is given as a fair illustration of the results ob-

TABLE 1

PATIENT				RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
No.	Sex	Age	Dental condition	Alkali	Acid	T. N.	Alkali	Acid	T. N.	
		years								
48306	M	11	Immune with care	3.5	6.1	9.6	0.3	19.7	20.0	48.00
48367	F	11	Immune	1.3	3.5	4.8	1.6	5.5	7.1	67.60
48234*	M	14	Immune	1.4	3.3	4.7	0.8	9.7	10.5	44.76
48291*	F	15	Immune with care	3.8	6.2	10.0	0.7	23.0	23.7	42.11
48148	F	12	Immune with care	1.0	4.5	5.5	0.4	13.2	13.6	40.44
48214	M	13	Immune	1.1	3.1	4.2	1.0	10.3	11.3	37.16
45928	F	13	Immune with care	3.0	2.1	5.1	0.6	4.1	4.7	108.50
45642	F	12	Immune with care	4.4	7.5	11.9	1.4	19.5	20.9	56.93
1962P*	M	14	Immune	5.8	15.7	21.5	2.7	35.2	37.9	56.72
1962S†	F	12	Immune with care	0.4	3.7	4.1	0.3	8.5	8.8	46.66
45861*	F	10	Immune with care	0.3	5.5	5.8	0.6	13.4	14.0	41.42
48569	F	14	Immune with care	1.7	2.4	4.1	1.1	7.6	8.7	47.12
Averages				2.88	5.3	7.35	0.9	14.1	15.0	52.43

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

tained from immune mouths. The columns headed "alkali" mean the amount of alkali used to neutralize the sample or the acidity of the sample. "Acid" designates the amounts of acid used or the alkalinity of the sample.

No constancy of results can be detected in the preceding table. The figures given for acidities, alkalinities, total neutralizing powers and salivary factors vary widely.

For example, the acidity column of resting saliva gives outside limits of alkali used as 5.8 cc. and 0.3 cc. with the average 2.88 cc. Wider variations than these are found in all but the acidity column of activated saliva.

TABLE 2

PATIENT				RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
No.	Sex	Age	Dental condition	Alkali	Acid	T. N.	Alkali	Acid	T. N.	
		years								
47084K*	F	14	Decay	5.5	3.7	9.2	2.5	3.1	5.6	164.26
47084P	F	11	Decay with care	0.7	1.8	2.5	0.5	7.7	8.2	30.48
48374	M	11	Decay	0.7	2.0	3.7	0.2	2.4	2.6	103.82
8431H	F	14	Decay	1.2	4.4	5.6	0.6	4.5	5.1	109.01
19564*	F	12	Decay with care	3.2	5.4	8.6	0.7	20.8	21.5	40.00
9732TP*	F	13	Decay	1.2	2.4	3.6	0.8	5.3	6.1	69.01
8362	M	12	Decay with care	1.1	2.8	3.9	0.6	7.2	7.8	50.00
1906A	M	12	Decay with care	0.4	2.7	3.1	0.5	4.5	5.0	62.00
46873†	M	13	Decay	2.2	3.7	5.9	0.1	6.4	6.5	90.76
1908A	M	12	Decay with care	1.1	5.2	5.3	0.1	10.7	10.8	49.07
1965A†	M	12	Decay	0.3	1.4	1.7	0.1	4.4	4.5	38.00
Averages				1.66	3.22	4.82	0.6	7.7	8.3	64.96

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

Table 2 shows the results from carious mouths.

This table shows a similar disregard for constancy. An example of the inconstancy here is the salivary factor column, where the average factor is 64.96, and one of 164.26 and one of 38.00 are found with variations from these in between.

Comparison of tables 1 and 2 displays no striking difference. The averages of all columns of the immune table except that of salivary factors are higher than those of the caries table, but in every column of the immune cases there will be found figures below the average of the corresponding column of carious cases and likewise in every column of the caries table there will be found figures above the average of the corresponding column of immune cases. Table 3 illustrates this.

TABLE 3

CASE NO.	DENTAL CONDITION	RESTING SALIVA			ACTIVATED SALIVA			SALIVARY FACTOR
		Alkali	Acid	T. N.	Alkali	Acid	T. N.	
47084	Decay	5.5	3.7	9.2	2.5	3.1	5.6	164.26
19564	Decay with care	3.2	5.4	8.6	0.7	20.8	21.5	40.00
48214	Immune	1.1	5.3	4.2	1.0	10.3	11.3	37.16
48928	Immune with care	3.0	3.22	5.1	0.6	4.1	4.7	108.50
1962S	Immune with care	0.4	3.1	4.1	0.3	7.5	8.8	46.66
45642	Immune with care	4.1	2.1	11.9	1.4	19.5	20.9	56.93
Average	Immune	2.88	3.7	7.35	0.9	14.1	15.0	52.43
Average	Decay	1.66	7.5	4.82	0.6	7.7	8.3	64.93

This table also illustrates the fact that while the average salivary factor for carious mouths is higher than that of immune mouths, both factors are below 80+ (Marshall's line of demarcation between an immune and carious mouth), and again that factors of carious cases may be below the average factor of immune cases and factors of immune cases may be above the average carious factor.

Some samples of saliva, both resting and activated, were alkaline to phenolphthalein, but this phenomenon occurred with such frequency in both immune and carious cases that no importance can be attached to it. Table 4 shows the indiscriminancy with which it occurred. The resting or activated or both kinds of saliva of all of these examples were alkaline to phenolphthalein.

TABLE 4

CASE NO.	AGE	DENTAL CONDITION	SALIVARY FACTOR
	years		
48234*	14	Immune	44.76
47084K*	14	Decay	164.26
1962S†	12	Immune with care	46.66
19564*	12	Decay with care	40.00
46873†	13	Decay	90.76

* Activated saliva alkaline to phenolphthalein.

† Activated and resting saliva alkaline to phenolphthalein.

The following table shows very well the lack of constancy of sex and age in regard to the salivary factor. Numbers 48306 and 48569, a boy and a girl with three years difference in age, vary hardly at all, and numbers 1906A and 1905A, both boys of the same age, give very different salivary factors.

TABLE 5

CASE NO.	SEX	AGE	DENTAL CONDITION	SALIVARY FACTOR
		years		
48306	M	11	Immune with care	48.00
48569	F	14	Immune with care	47.12
1906A	M	12	Decay	62.00
1905A	M	12	Decay	38.00

The general appearance of all the subjects was that of ordinary, normal boys and girls. Slovenly appearance was not displayed more frequently in one sort of a case than in the other. Carious mouths were found in both overdeveloped and underdeveloped as well as in normal children. Age, sex and condition of the mouth had no connection with the nervous embarrassment of the patient or the resulting salivary factor.

Table 6 gives examples of the inconstancy of salivary factors with regard to general appearance.

TABLE 6

NATIONALITY	CASE NO.	AGE	SEX	DENTAL CONDITION	SALIVARY FACTOR
		years			
Swede	19564	12	F	Decay with care	40.00
French	9732TP	13	F	Decay	59.00
Irish	8362	12	M	Decay with care	50.00
Irish	47084K	14	F	Decay	164.26
Irish	48148	12	F	Immune with care	40.42
Jewish	48928	13	F	Immune	108.50

The first two cases cited were unusually attractive, healthy, wholesome looking girls, but they had atrociously bad teeth. Each girl had six sound teeth in her head. Number 8362, who also had very poor teeth was a sickly undernourished little fellow who displayed great embarrassment. Of the same general nervous appearance was 48148, but in this instance she had a perfect set of teeth. The two with factors above 80 were normal appearing, with no distinctive characteristic except that one had excellent and the other poor teeth.

All nationalities were represented in our cases. Irish children abounded, with Jewish and French as close seconds. There was one colored girl with an immune mouth. These nationalities were placed in both immune and carious tables.

A glance at any of the tables displays the fact that care of the teeth played no part in either the dental condition or the salivary factor. There was sometimes doubt with both immune and carious cases as to the veracity of their acknowledgment of care.

Every sample of saliva was collected at least two hours after a meal. The character varied considerably, but not consistently. Table 7 shows this. The activated saliva of every patient was darker colored, less viscid and secreted more rapidly than the resting saliva.

TABLE 7

CASE NO.	DENTAL CONDITION	CHARACTER OF SALIVA	SALIVARY FACTOR
47084K	Decay	Evil smelling, bloody, viscous, secreted slowly	164.26
47367	Immune	Dark, viscous, secreted slowly	67.60
1905A	Decay	Colorless, clean, secreted rapidly	38.00
1908A	Decay with care	Opaque, secreted with average rapidity	49.07

SUMMARY

From observations made on these cases, it would appear that the saliva of persons with teeth immune to caries varies, as does also the saliva of persons with carious teeth; that saliva may neutralize substances taken into the mouth and that the average immune mouth has the greater power of neutralization; but the ratio of resting and activated saliva in immune mouths does not vary enough from that of carious mouths to prove that this ratio is indicative of the production and maintenance of immunity from caries in any individual.

The tables compiled do not show consistently that as the difference between the total neutralizing powers of resting and activated saliva diminishes, liability to incidence of caries increases.

Since the average ratio or "salivary factor" is below 80 in both immune and carious mouths, there is doubt as to the importance of this mark in the relation of the salivary factor and dental caries.

Furthermore, no constant points of difference can be found to correspond with the differences in salivary factors and in our work we can find no substantial proof to verify a relationship of the salivary factor to dental caries.

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THE INFLUENCE OF MUSIC UPON ELECTROCARDIOGRAMS AND BLOOD PRESSURE

I. H. HYDE AND W. SCALAPINO

From the Physiological Laboratory of the University of Kansas

Received for publication February 4, 1918

The object of the experiment of which only the beginning is here briefly summarized, is to ascertain the effect of different kinds of music upon the heart and blood pressure in individuals who are known to have musical talent and are fond of music; also in persons who are indifferent and have no fondness for music, in neurasthenics and in some animals.

The cardiograms were recorded with the Einthoven string galvanometer, its sensitivity was 1 cm. deflection per one millivolt; the film speed was 2.5 cm. per second. The time marker recorded ⅕ second, and lead 2 was adopted for comparison, for all of the records.

The pulse and pressure were obtained with a Tycos and a modified form of Erlanger's sphygmomanometer, and the music from Victrola records.

The pieces of music selected were first, Tschaikowsky's death symphony, characterized by its tragic slow minor movements; and second, the Toreador's brilliant description of the bull fight from Carmen; and third, the National Emblem, a stirring rhythmical march by Sousa. The effects of other pieces of music had been tested but the results seemed to indicate that the effects of those pieces that were familiar to the subjects, were influenced also by associated memory.

The data from subject "A" who is fond of music and whose voice has been cultivated, were checked up with those of two other subjects, but his being most complete were chosen for presentation.

The experiments were conducted under fairly constant subjective and weather conditions, and about the same hours of the day. The cardiograms, pulse and pressure curves were secured before and immediately after, and also from five to ten minutes after the music had ceased. But while listening to the music, only the cardiograms were taken because it seemed that the latter were affected by the manipulations necessary to secure blood pressure records. More experiments are needed before it is possible to state how long the after effects of the music persist, and also to ascertain if different kinds of quality or timbre may have different effects. The same piece of music if sung or played by the orchestra or piano or violin might have a different influence. For control data, the subjects' cardiograms, pulse and pressure were obtained, without the influence of music, at different hours of the morning, the forenoon being the time during which the tests were all made. It was found that ordinarily the pulse rate and pressure vary somewhat during the forenoon as shown in table 1.

The fact that the pulse rate decreases and the pulse pressure increases as the morning advances must therefore be taken into consideration in estimating the after effects of music.

From a study of the table, we learn that while listening to the symphony, the average effect is a slight decrease if any of the "P. P." wave, and therefore a relatively slight increase in the pulse rate, and also that the amplitude or E. M. F. of the "R" wave is increased. We find also, that from two to ten minutes after the music has ceased, the pulse rate and the E. M. F. have increased considerably, but the systolic and pulse pressure have fallen.

TABLE 1

Mean results of the morning variations: in pulse and pressure

TIME	PULSE PER MINUTE	SYSTOLIC PER MILLIMETER	DIASTOLIC PER MILLIMETER	PULSE PRESSURE PER MILLIMETER
10.45	84	112	76	36
11.15	82	114	76	38
11.30	78	114	74	40
11.45	76	114	72	40
12.00	72	114	74	40

Consequently the minor tones of the symphony records caused an increase in cardiac activity and action current, but a fall in blood pressure. The increased pulse rate and decreased blood pressure are probably the result of psychic or reflex inhibition of the vagus nerve and vasomotor center. The shortening of the "P. P." wave is mainly due to the decrease of the "T. P." wave, or pause, of the cardiac cycle.

Toreador's stirring song produced a different picture of cardiograms, seen by an inspection of table 2. We find that the pulse rate was accelerated and the E. M. F. or amplitude of "R" wave became less as soon as the song was heard. This is graphically shown in the decrease of the "P. P." and "T. P." phases, and height of the "R" wave in cardiograms obtained while listening to the song. The after effect was increased systolic and pulse pressure and pulse rate, but decreased action current. It seems, therefore, that this kind of music had a stimulating effect upon the circulation by increasing the blood pressure and pulse rate while lessening the action current of the ventricular contraction. This change may be due to reflex action of the accelerator nerve or possibly inhibition of the vagus.

TABLE 2

Summary of cardiograms and pressure

MUSIC	CURVE	TIME	RELATIVE TO MUSIC	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE PER MINUTE	"P.P." PER CUBIC MILLIMETER	"T.P." PER CUBIC MILLIMETER	"E.M.F." AMPLITUDE "R"
				mm.	*mm.*	*mm.*				
Symphony........	54	11.15	Before	110	65	45	76	1.98	0.82	0.50
	55	11.20	During				83	1.80	0.72	0.55
	56	11.25	During				75	2.00	0.85	0.56
	57	11.30	During				73	2.03	0.90	0.55
	58	11.32	After	102	60	42	80	1.86	0.74	0.58
	59	11.36	After	102			81	1.84	0.71	0.58
Toreador..........	46	11.00	Before	106	78	28	81	1.84	0.77	0.60
	47	11.5	During				83	1.80	0.68	0.58
	48	11.10	During				87	1.71	0.58	0.56
	49	11.15	After	112	74	37	85	1.76	0.65	0.57
National March...	41	11.40	Before	106	70	36	75	2.00	0.80	0.60
	42	11.50	During				70	2.14	0.90	0.62
	44	11.57	During				71	2.09	0.85	0.63
	45	12.00	After	112	66	56	75	2.10	0.84	0.70

1 cm. per millivolt. film speed 2.5 cm. per second. Time ⅕ second. Lead 2.0 for all records.

The effect of the inspiring rhythmical tones of the National Emblem, as seen from table 2, was a slower pulse rate, a longer pause or "T. P." wave, and an increase of not only the systolic and pulse pressure but also the action current of the ventricular contraction. It seems that this music had its stimulating effect upon the vagus, and that this as well as other kinds of music may have an influence on the system in other respects. It very likely affects digestion, secretion, muscle tone, and respiration. But many more experiments are needed before definite conclusions can be drawn. A survey of the preliminary results obtained with the three classes of music indicates that in the subjects experimented on, the minor tones of music increased the pulse rate and action current of the ventricular contraction, and lowered the systolic and diastolic pressures. On the other hand, the stirring notes of Toreador's song, and also those of the rhythmical march, increased the systolic and pulse pressure, but the former also increased the pulse rate, with decreased diastolic pressure and action current, while the march slowed the cardiac cycle and increased its action currents. It is possible that a careful selection of music may be a beneficial aid in the treatment of nervous disturbances.

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THE REGULATION OF RENAL ACTIVITY

IV. Regulation of Urea Excretion by Adrenalin

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The general conditions we have already outlined (1) were observed. No food or water was given for about seventeen hours before the commencement of the experiment, which lasted for five hours and began about 9 a.m. During this time four collections of urine and of blood were made. No urea was given, but in order to keep the conditions uniform with those in other experiments the stomach tube was passed just before the bladder was first washed out. This procedure was carried through on a group of twenty-eight rabbits. The experiments were then repeated on the same animals under the same conditions except that 0.25 cc. of Parke, Davis & Co.'s 1-in-1000 Adrenalin Chloride was injected subcutaneously at the commencement of each of the five hours of observation. The average rates of excretion, blood urea concentrations and ratios for each of the four periods of the experiment without and with adrenalin, are given in table 1 and charted in figure 1. The details for the individual animals will be found in table 6 at the end of the paper.

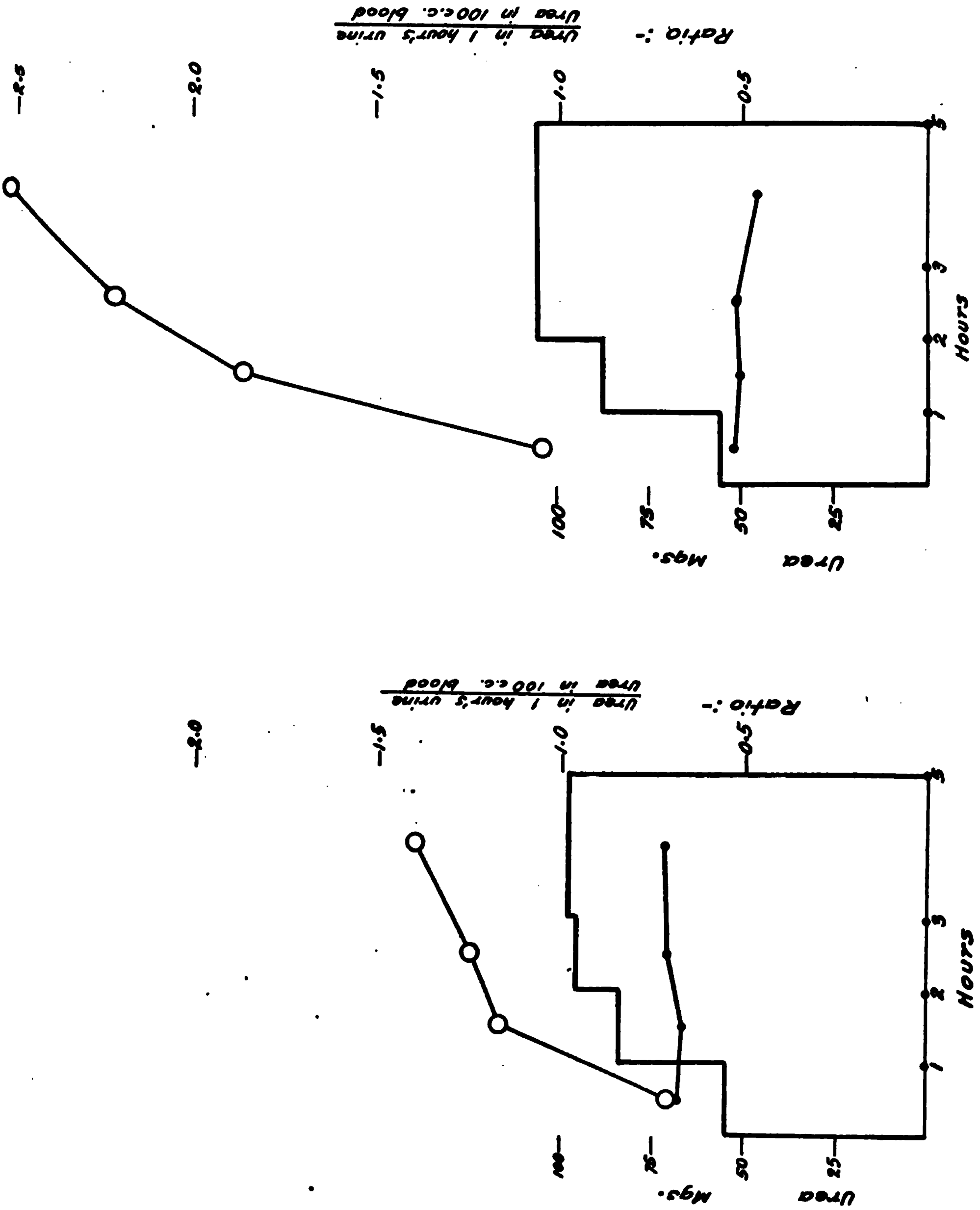
The five subcutaneous injections of 0.25 cc. of 1-in-1000 adrenal solution increase the rate of urea excretion and at the same time decrease the blood urea concentration. There is, consequently, a marked increase in the ratio between the urea content of the urine and of the blood. It should be noted that the decrease in the blood urea concentration is a circumstance which in itself should have tended to lower rather than raise the ratio (2).

Reference to table 6 will show that there is a considerable degree of variation in the manner in which individual animals react to adrenalin. If these figures are compared with the control experiments without adrenalin given in the preceding paper of this series, it will be found that in some cases the increase in the ratio is very marked, while in others it is only slight. It will be noted also that there are occasional instances in which, in one period or another, the ratio is greater without than with adrenalin. It is, therefore, necessary to determine whether the actual differences noted between the average ratios without and with adrenalin might not be due to chance. If both sets of experiments were to be repeated many times a series of averages would be obtained which would differ somewhat from those we obtained. It might be found then that we had chanced to get an unusually low average without adrenalin and an unusually high average with adrenalin, so that the difference we found was atypical and misleading. It is possible, however, to calculate from our data the "probable error" of each average and from this to obtain the "probable difference between the averages." These are given for each period in table 1. They indicate the value of the difference which would include half of all the differences between the averages without and with adrenalin which would be encountered if the experiments were repeated many times. It is clear from the fact that the probable differences are much smaller than the actual differences, that it is very unlikely that the latter are due to chance. In the case of the fourth period averages, reference to Davenport's tables (3) shows that there is only one chance in over one hundred thousand that accidental variation could bring about such a difference as actually occurred. But it is also necessary to take into considera-

TABLE 1

Comparison of averages from a group of 28 rabbits without and with 0.25 cc. adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.25 CC. ADRENALIN			ACTUAL DIFFERENCES BETWEEN RATIO AVERAGES	"PROBABLE DIFFERENCES" BETWEEN RATIO AVERAGES
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	55	68	0.71	56	52	1.04	+0.33	±0.69
II	84	67	1.17	88	51	1.86	+0.69	±0.12
III	96	71	1.27	106	52	2.21	+0.94	±0.16
IV	98	72	1.40	106	46	2.52	+1.12	±0.18



With 0.25 cc. adrenalin

Without adrenalin

Fig. 1

tion the fact that in all four periods the difference is in the direction of an increased average after adrenalin. This fact so reduces the possibility of accounting for the actual differences on the basis of chance that it becomes inappreciable. There can then be no question but that the rise in the plane of renal efficiency illustrated in the higher level of the ratio curve after adrenalin administration is a specific effect of the adrenalin itself.

The effect of adrenalin varies with the quantity injected. In figure 2 and figure 3 the averages of small groups are charted without and with doses of 0.125 cc. and 0.0625 cc. of 1-in-1000 adrenalin. The effect of the adrenalin does not become apparent until the fourth period. The degree of increase in the activity of the kidney, therefore, decreases as the amount of adrenalin injected is decreased.

TABLE 2

Comparison of a group of 6 rabbits without and with 0.125 cc. adrenalin

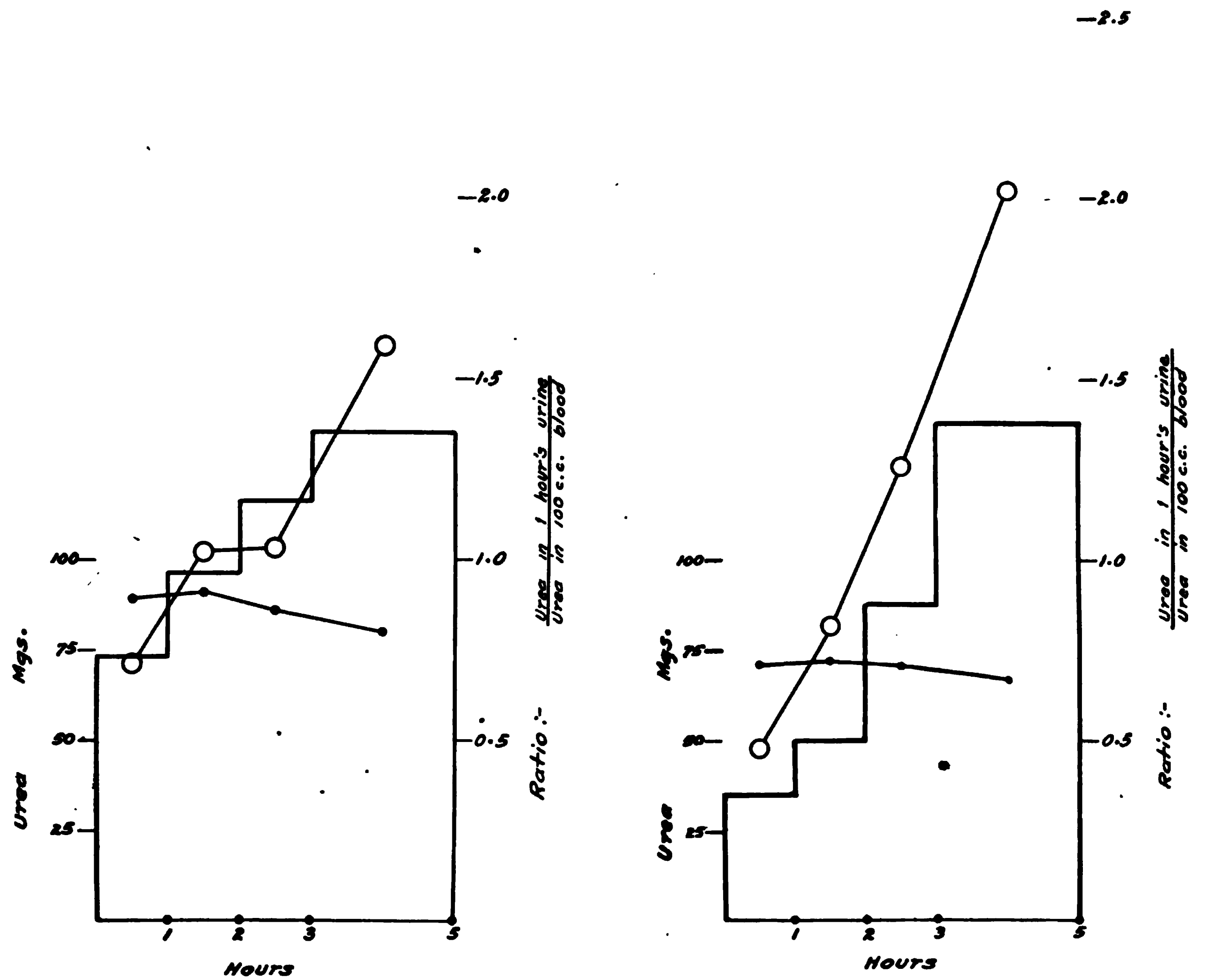
PERIOD	WITHOUT ADRENALIN			WITH 0.125 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	73	89	0.71	35	71	0.48
II	96	91	1.02	50	72	0.82
III	116	86	1.19	88	71	1.26
IV	135	80	1.59	138	67	2.02

TABLE 3

Comparison of a group of 7 rabbits without and with 0.0625 cc. adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.0625 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	48	61	0.74	18	42	0.42
II	74	62	1.18	35	41	0.84
III	92	64	1.45	59	44	1.40
IV	102	67	1.56	66	42	1.65

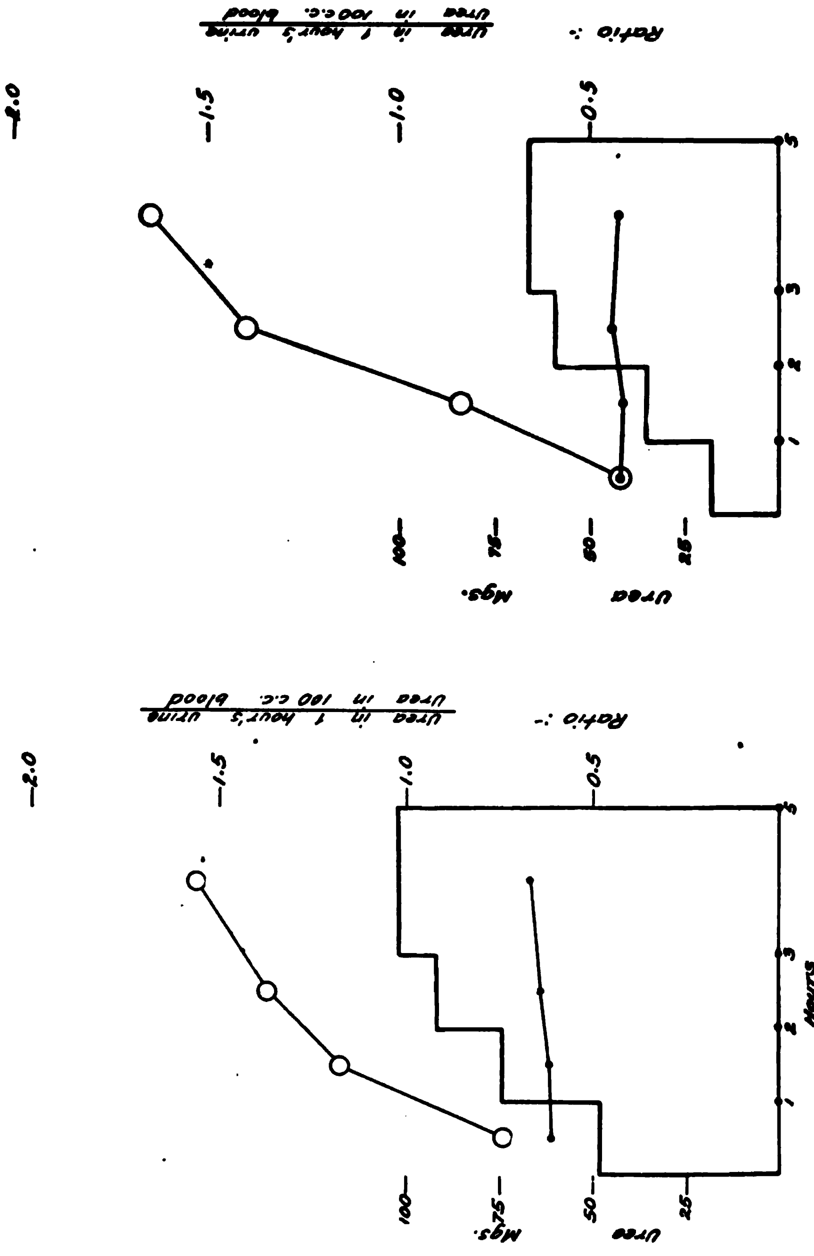
An interesting result was obtained by increasing the amount of adrenalin. The effect of 0.5 cc. on a group of rabbits is shown in figure 4. It will be noted that the increase in the ratio is less than after 0.25 cc. The averages of three rabbits which were given 1 cc. at the



Without adrenalin

With 0.125 cc. adrenalin

Fig. 2



With 0.0625 cc. adrenalin

Without adrenalin

Fig. 3

beginning of the first hour and 0.5 cc. at each of the four succeeding hours are charted in figure 5. Here adrenalin produces an effect which is directly contrary to that obtained with smaller doses. The decrease in the ratio is so pronounced that it would seem likely that it represents the renal reaction to the generalized toxic effect described by Elliott (4) as following large amounts of adrenalin.

TABLE 4

Comparison of averages from a group of 13 rabbits without and with 0.5 cc. of adrenalin

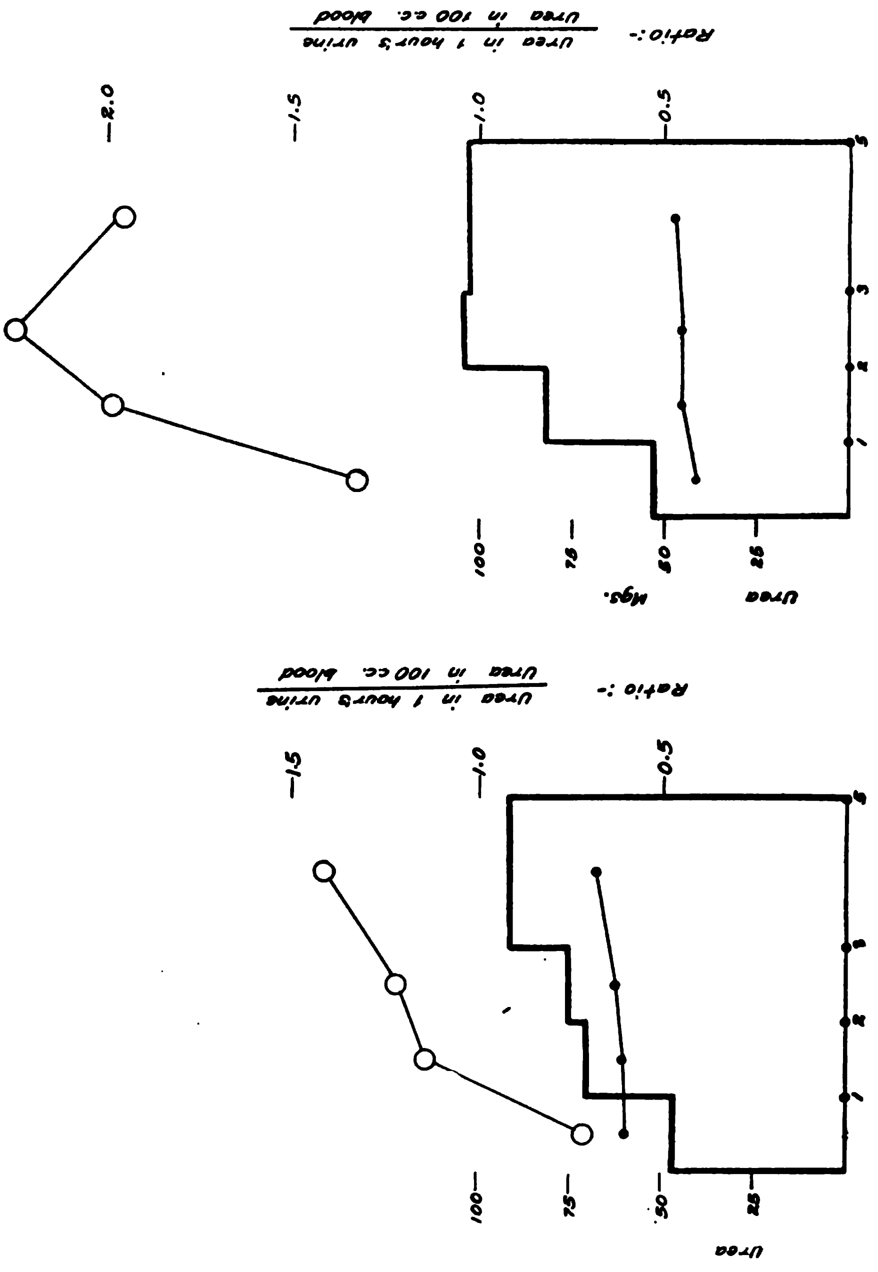
PERIOD	WITHOUT ADRENALIN			WITH 0.5 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	47	60	0.71	53	41	1.33
II	71	61	1.14	82	45	1.99
III	76	63	1.22	109	45	2.25
IV	92	68	1.41	109	47	1.96

TABLE 5

Comparison of a group of 3 rabbits without and with 0.625 cc. adrenalin (1 cc. at the commencement of period 1, and 0.5 cc. thereafter)

PERIOD	WITHOUT ADRENALIN			WITH 0.625 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	30	46	0.63	31	81	0.37
II	53	47	1.15	36	85	0.41
III	66	48	1.40	49	93	0.58
IV	96	50	1.93	54	95	0.60

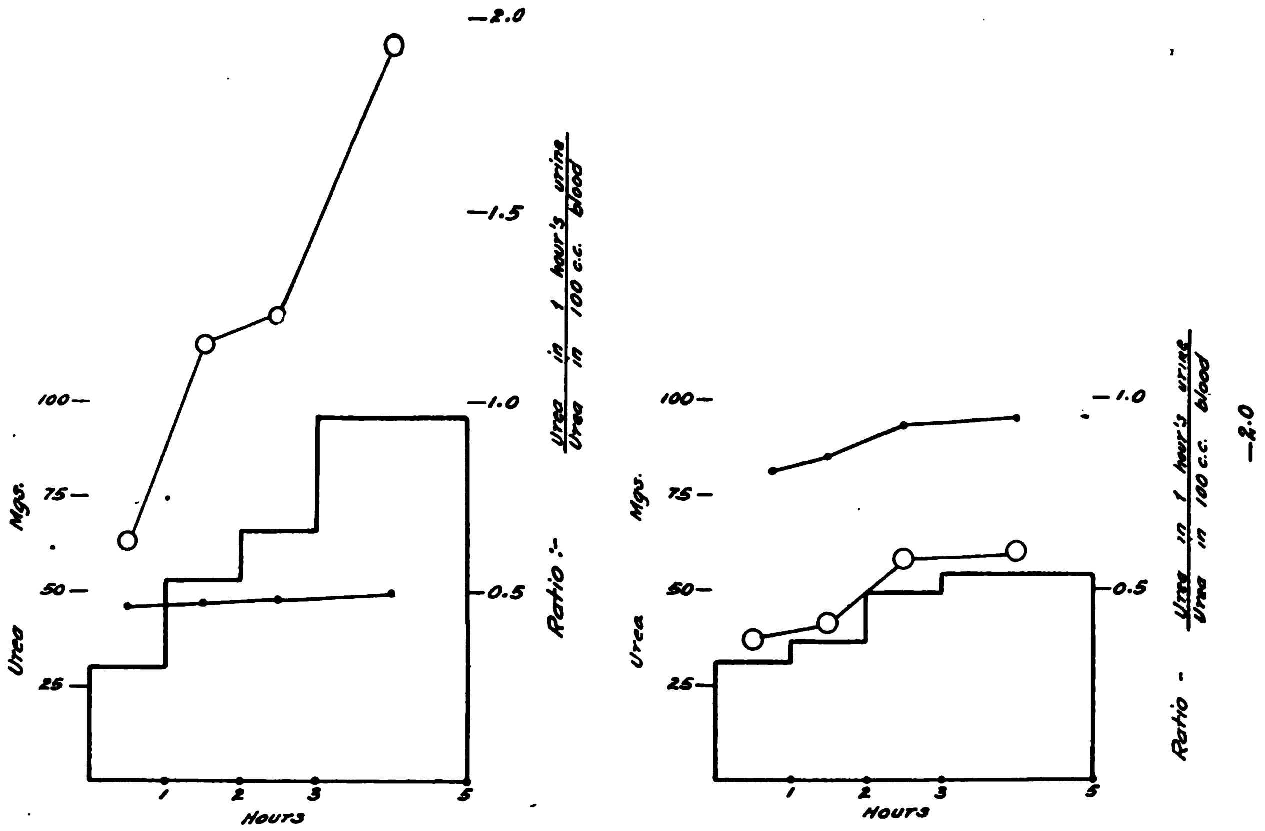
Although the question as to the exact quantities of adrenalin effective in regulating the rate of urea excretion could be accurately decided only by slow long-continued intravenous injections, our results at least indicate that a gradation of renal stimulation is produced by increasing doses, that there is an optimal quantity which leads to a great increase in kidney action and that amounts above this optimum have less effect until, with relatively large quantities, an actual depression of kidney function results.



With 0.5 cc. adrenalin

Without adrenalin

Fig. 4



Without adrenalin

With 0.6 cc. adrenalin

Fig. 5

TABLE 6

Adrenalin 0.25 cc. hourly

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:
59	54	60	0.90	72	54	1.33	18	54	0.33	84	51	1.63
65	151	58	2.60	105	65	1.62	124	65	1.91	137	51	2.70
66	49	39	1.25	52	21	2.46	80	63	1.27	lost	lost	lost
67	32	57	0.57	104	57	1.84	133	54	2.47	130	51	2.55
71	68	45	1.52	97	30	2.95	110	51	2.15	49	51	0.96
72	lost	lost	lost	109	51	1.58	158	54	2.93	128	51	2.50
80	188	153	1.23	214	114	1.88	333	102	3.27	246	93	2.64
82	176	174	1.01	189	174	1.09	202	177	1.14	lost	lost	lost
85	28	27	1.03	46	33	1.39	54	21	2.57	78	21	3.71
86	5	30	0.15	28	30	0.95	39	21	1.86	143	23	6.20
87	45	54	0.83	126	60	2.10	210	67	3.14	120	66	1.82
88	53	69	0.77	100	69	1.45	137	64	2.14	131	63	2.08
90	46	26	1.79	93	28	3.32	86	35	2.44	114	34	3.34
93	30	45	0.68	59	48	1.23	75	48	1.56	134	45	2.98
94	49	47	1.04	75	48	1.56	70	48	1.45	65	50	1.31
95	41	51	0.80	77	53	1.45	70	66	1.07	51	69	0.74
96	23	33	0.71	40	35	1.16	53	30	1.75	34	36	0.94
97	42	45	0.93	63	33	1.91	54	44	1.22	249	42	5.94
98	43	39	1.12	26	39	0.65	73	36	2.04	90	36	2.50
99	32	40	0.81	65	42	1.51	96	33	2.91	99	35	2.83
100	29	33	0.89	81	35	2.32	49	36	1.37	40	35	1.15
102	57	45	1.27	107	44	2.44	117	45	2.60	66	48	1.36
103	38	39	0.94	109	24	4.57	124	18	6.88	124	24	5.16
104	53	56	0.95	120	63	1.90	120	68	1.77	103	69	1.50
105	30	30	1.00	47	28	1.69	43	27	1.58	47	27	1.75
68	50	36	1.38	85	45	1.90	119	44	2.70	114	45	2.53
70	16	34	0.49	51	39	1.31	70	40	1.75	82	42	1.96
73	77	52	1.47	133	54	2.46	141	39	3.62	112	42	2.68
Averages............	56	52	1.04	88	51	1.86	106	52	2.21	106	46	2.52
Averages for the same rabbits without adrenalin................	55	68	0.71	84	67	1.17	96	71	1.25	98	72	1.40
The amount by which ratios obtained with adrenalin exceed the average ratios without adrenalin...............			+0.33			+0.69			+0.96			+1.12

DISCUSSION

We have shown that the injection into the subcutaneous tissues of certain amounts of adrenalin is followed by a marked increase in the activity of the kidney in the excretion of urea. This fact raises the question as to whether the adrenin produced by the suprarenal glands within the body may not be one of those unknown factors in the regulation of renal function whose mode of action was discussed in the preceding paper of this series. To those who are conversant with the recent literature on adrenin this possibility may at first sight seem remote. The work, especially, of Stewart and Rogoff has failed to confirm the validity of theories under which certain physiological phenomena were related to variations in the rate of adrenin secretion. And it will be objected that we do not even know that there is any adrenin in the blood as it reaches the kidney, since sensitive involuntary muscle test-objects fail to show its presence in blood from the jugular vein (5). Nevertheless, we hold that it would be premature to assume that adrenin produced within the body may not play a part in the regulation of renal function. For apart from the possible route by which adrenin may reach the kidney directly, (6) there is, as we shall show, good reason to believe that the secretory activity of the kidney may be influenced by amounts of adrenin much smaller than those required to alter the action of involuntary muscle fibers. This question of the relation between adrenin and renal function will be more fully discussed in a later paper.

So much attention has been paid to the effect of adrenalin on muscular action that it is natural at first to suppose that its stimulating effect on the kidney is secondary to changes induced in the circulatory conditions within the organ. But the evidence as to the action of adrenalin on the renal vessels goes to show that it decreases the rate of flow of blood through the kidney. Hoskins and Gunning (7) have recently investigated the effect of the intravenous injection of both depressor and pressor doses. In every one of seventeen experiments they found the rate of flow of blood from the cut renal vein to be diminished. There would thus be an apparent contradiction in the vascular and secretory effect of adrenalin, if there were reason to believe that adrenalin given subcutaneously, as in our experiments, had any effect on the renal artery. There is, however, no such reason. When given subcutaneously the rate at which adrenalin gains entrance to the blood stream is very slow (4). In the rabbit Biedl (8) was not able to obtain a rise of blood pressure with any dose given subcutaneously. Bilberfeld (9) found no effect on the blood pressure of rabbits after the subcutaneous injection of 2 to 5 mgm. of adrenalin. These are enormous quantities as compared with those which have a marked effect on blood pressure when given intravenously. Yet though the rate of absorption of adrenalin from the tissues is so slow that at any one time there is apparently never a sufficient concentration in the blood to produce arterial effects, we do know, from the occurrence of such a phenomenon as glycosuria, that some adrenalin reaches the blood stream. Certain adrenalin effects may thus result from quantities of a different order of magnitude from those required to produce circulatory effects. Further it must be remembered that even if it were found that a greater amount of blood passed through the kidney during the period in which a hypersecretion of urea occurs after the subcutaneous injection of adrenalin, it would still remain to be proved that the circulatory changes were the cause and not the result of the increased renal activity. For these reasons we think it highly improbable that the augmenting action of adrenalin on the secretion of urea in our experiments is the result of an adrenalin action on the blood supply of the kidney.

Does adrenalin then act as a direct stimulant to the urea secreting components of the kidney cells? All analogy is against such a view. Adrenalin, for instance, does not act on the contractile elements of muscle cells but on the myo-neural junction,—the receptive substance of Langley (10)—Elliott's generalization (4) still stands that any tissue or organ responding to adrenalin is under the control of the sympathetic nervous system, though the converse that all organs innervated from the sympathetic are sensitive to adrenalin is not invariably true. But though the kidney is richly supplied with sympathetic nerves it has not been proved that they have any direct influence on the secretory activity of the kidney, because it has always been possible to ascribe the secretory changes which follow their stimulation to the concomitant circulatory alterations which this procedure also induces. And there seems to be a tendency to accept, almost as a proved fact, the conception that the kidney is not regulated through the nervous system except in an indirect manner through its blood supply. But it is noteworthy that there is conclusive anatomical evidence which runs counter to this view. All nerve fibers do not end in the blood vessels. The glomeruli and tubules are surrounded by a quite separate and distinct plexus of nerve fibers, which end upon or between the renal cells. This was first discovered by Berkley (11) in 1893 and it has since been repeatedly confirmed, most recently by Smirnow (13) and by Renner

(14). We, therefore, believe that adrenalin influences the secretory activity of the kidney cells through the medium of something in the termination of sympathetic nerve fibers analogous to the receptive substance in the end-plates of muscle fibers.

CONCLUSION

The subcutaneous injection of adrenalin (Parke, Davis & Co.) is followed by an increase in the urea excreting activity of the rabbit's kidney. There is a certain amount of adrenalin which produces the greatest increase in function. Smaller amounts have less and less effect until there is no change from the normal. With larger amounts the augmenting effect on secretion also becomes less until, with relatively large doses, the reverse effect of a decrease in function is found. Except with these large amounts the rate of urea excretion is more rapid than in animals not given adrenalin, in spite of a lowering of the blood urea concentration.

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THE REGULATION OF RENAL ACTIVITY

V. Regulation of Urea Excretion by Pituitrin

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There is no conclusive evidence that the pituitary gland contains any substance which alters the activity of the kidney. It is true that in some pathological conditions in which the gland is involved, the volume of the urine is found to be greatly increased. It is also known that the volume of urine is increased by intravenous injection of pituitary extract, and is decreased by subcutaneous injection of the same extract. But though these facts are probably correctly explained on the assumption that some substance in the pituitary gland alters the activity of the kidney in the excretion of water, they may also be accounted for on the hypothesis that the changes in the amount of water excreted are the passive results of concomitant changes in the amount of water available for excretion. On this view the active principle in the pituitary extract might be a regulator of the amounts of water held or liberated by the tissues in general, rather than a factor controlling the capacity of the kidney cells to abstract water from the blood. A true regulation of the water excreting function of the kidneys would be proved only if it were shown that the excretion of water was changed by pituitary extract in a manner which could not be accounted for as a result of simultaneous alterations in the quantity of free water in the blood. Since there is no method by which we can measure that fraction of the total water content of the blood which is "free," the question as to which of these two explanations is correct cannot at present be decided.

But there is no difficulty of this sort in interpreting the meaning of changes in urea excretion. There is no "bound" urea in the blood. All is immediately available for excretion. And if we find that the administration of pituitary gland extract leads to any significant alteration in the rate of urea excretion which cannot be accounted for on the basis of a change in the urea concentration of the blood, we may conclude that it is the activity of the kidney itself which has been altered.

The substance in pituitary extracts which induces the changes in the volume of urine we have referred to is present in the Pituitrin of Parke, Davis & Co. This extract also causes those changes in blood pressure and in the contractions of surviving segments of intestine which are produced by extracts of the pars intermedia of the pituitary gland.

The same methods were used as in the work on adrenalin. The pituitrin was given every hour by subcutaneous injection to a group of rabbits, and the average results compared with the averages obtained from the same group of animals under the same conditions when no pituitrin was given.

The averages from a group of nine rabbits without and with 0.25 cc. of pituitrin are given in table 1 and charted in figure 1. The details are given in table 6 at the end of the paper.

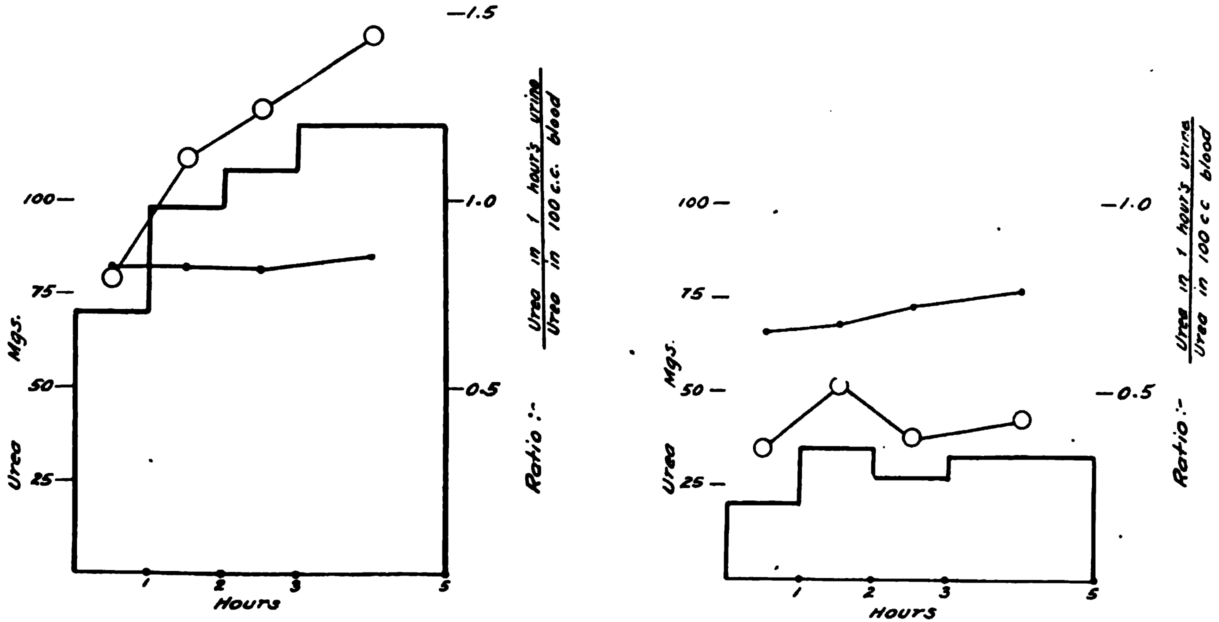
TABLE 1

Comparison of averages from a group of 9 rabbits without and with 0.25 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.25 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	70	82	0.79	20	66	0.35
II	98	82	1.11	35	68	0.52
III	108	81	1.24	27	73	0.38
IV	120	85	1.44	33	77	0.43

Pituitrin decreases the rate of urea excretion although an increase in rate would be expected since the blood urea concentration is higher than in the control experiments. The combination of a low rate of excretion with a high blood concentration results in a pronounced lowering of the ratio between the urea content of the urine and of the blood.

This is exactly the reverse of the effect of adrenalin, which increases the rate of urea excretion in spite of a reduction in the blood urea concentration. It seems probable that in both cases the change in the level of the blood urea concentration is the result of the altered rate of urea excretion. Both adrenalin and pituitrin would have changed the rate to a greater extent than they actually did if it had not been for



Without pituitrin

With 0.25 cc. pituitrin

Fig. 1

this secondary effect. The hyperactive kidney lowers the blood concentration and thus automatically diminishes one important stimulus to its activity, while a kidney whose function is depressed will, by its own inaction, more and more increase a factor which tends to awake it to renewed activity. The influence of those factors other than blood urea concentration which we have grouped under the term unknown, may thus be expected to be self limited and evanescent, since a state of excess of renal activity or of inactivity carries in itself the promise of its termination. It is therefore not strange that we should have found that deviations from the average reaction to any given level of blood urea concentration are temporary phenomena observable only over short time periods.

The remaining charts (figs. 2 to 5) show the effect of decreasing amounts of pituitrin. A few experiments were also carried out with larger and smaller doses. Amounts as high as 2 cc. gave results very similar to those obtained with 0.25 cc., and quantities between 0.005 cc. and 0.00001 cc. gave no certain result. We wished to see whether with very large or very small amounts there might not be a reversal of the usual effect such as was noted with adrenalin when large doses were given, but we did not multiply such experiments and they were discontinued as soon as we were able to conclude with a reasonable degree of certainty that pituitrin in all effective doses depressed the activity of the kidney. That a certain relation exists between the degree of depression and the amount of pituitrin given, is apparent from the charts.

TABLE 2

Comparison of averages from a group of 6 rabbits without and with 0.125 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.125 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	53	68	0.75	30	55	0.56
II	77	69	1.06	56	54	1.02
III	83	59	1.13	42	56	0.73
IV	106	75	1.52	46	60	0.77

TABLE 3

Comparison of a group of 6 rabbits without and with 0.0625 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.0625 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	53	68	0.74	34	57	0.46
II	77	69	1.05	62	55	0.90
III	83	69	1.18	46	58	0.63
IV	98	72	1.44	27	62	0.43

TABLE 4

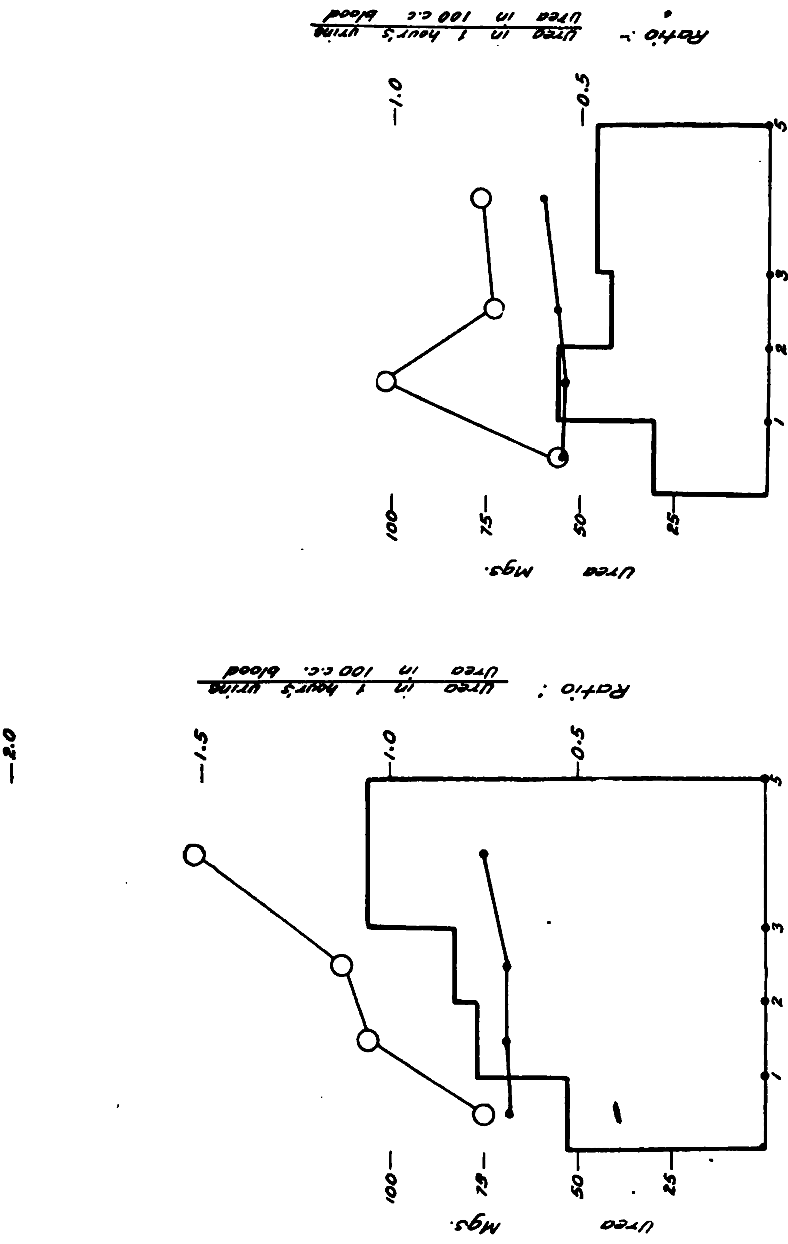
Comparison of averages from a group of 18 rabbits without and with 0.025 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.025 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	50	62	0.75	44	74	0.76
II	69	58	1.16	67	69	1.05
III	83	65	1.24	61	73	1.00
IV	92	64	1.46	73	77	1.00

TABLE 5

Comparison of averages from a group of 4 rabbits without and with 0.0125 cc. pituitrin

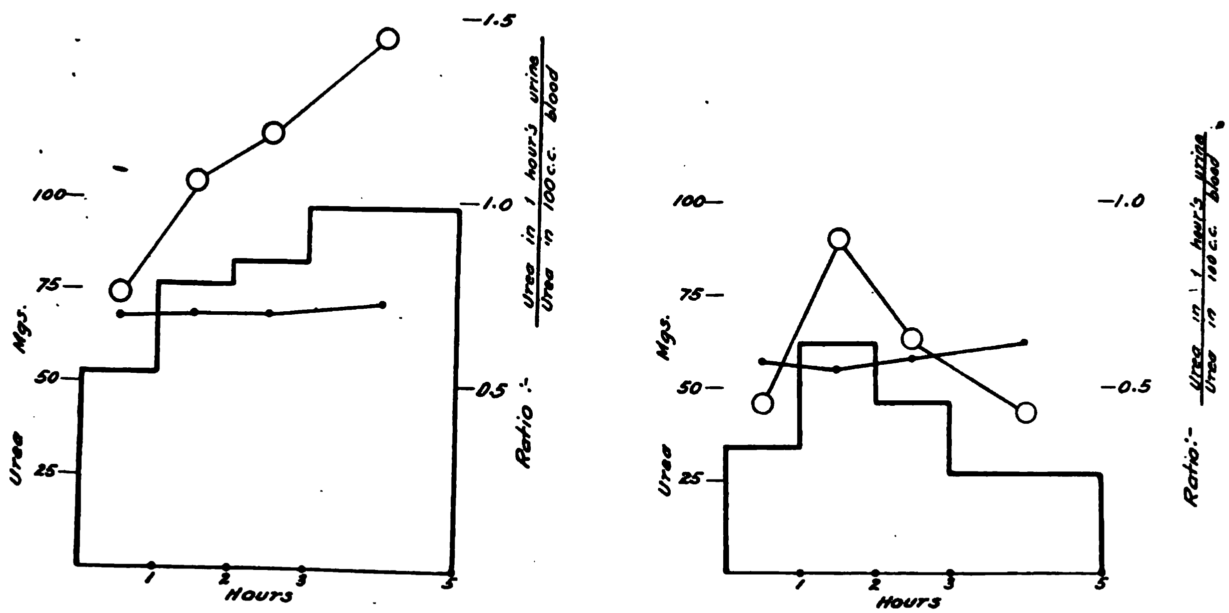
PERIOD	WITHOUT PITUITRIN			WITH 0.0125 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	31	90	0.41
II	90	74	1.19	45	94	0.53
III	96	74	1.28	51	95	0.54
IV	113	77	1.48	76	91	0.90



Without pituitrin

With 0.125 cc. pituitrin

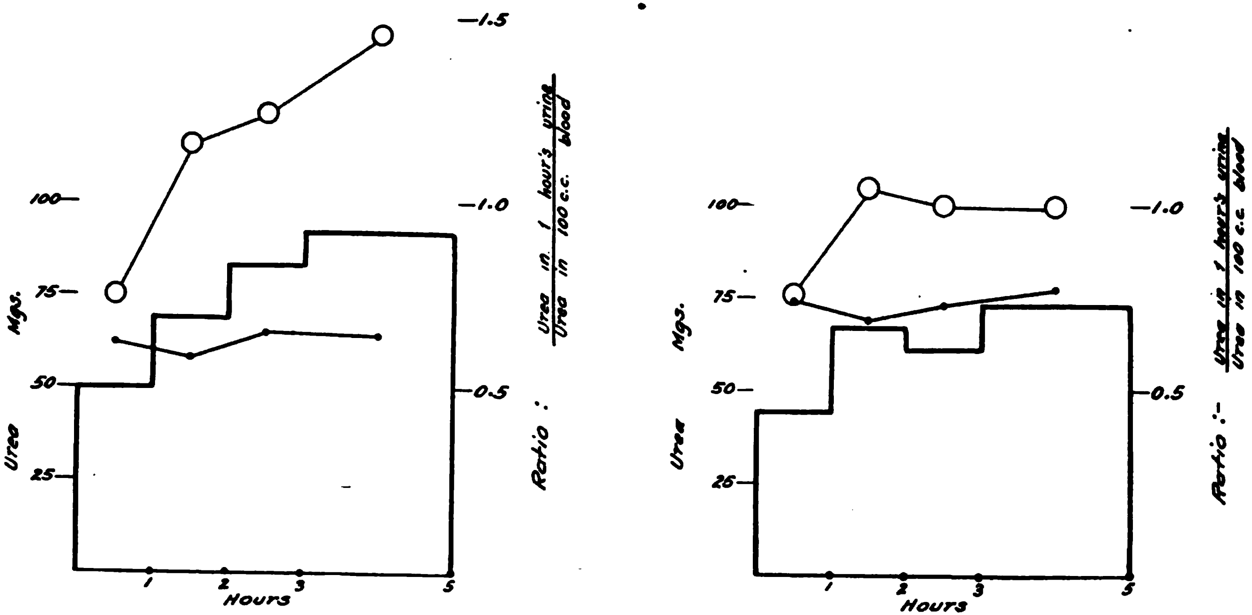
Fig. 2



Without pituitrin

With 0.625 cc. pituitrin.

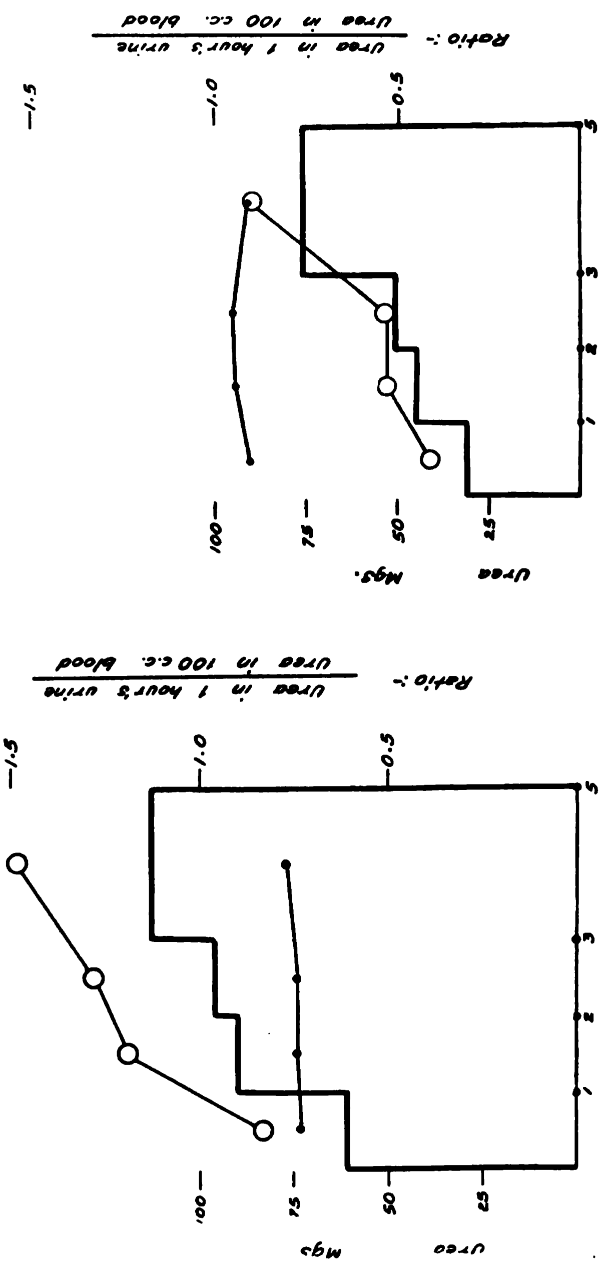
Fig. 3



Without pituitrin

With 0.025 cc. pituitrin

Fig. 4



With 0.0125 cc. pituitrin

Without pituitrin

Fig. 5

TABLE 6

Pituitrin 0.25 cc. hourly

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio
59	0	46	0.00	18	60	0.29	23	76	0.30	24	63	0.38
65	30	38	0.81	30	37	0.80	34	36	0.95	28	36	0.76
66	12	93	0.13	17	66	0.25	17	59	0.24	31	84	0.38
67	8	61	0.13	19	63	0.30	5	64	0.08	2	64	0.05
71	15	75	0.20	0	75	0.00	2	84	0.03	16	93	0.17
72	33	60	0.55	60	90	0.66	55	96	0.58	90	105	0.86
85	50	55	0.91	59	60	0.98	7	66	0.11	21	75	0.28
86	22	79	0.27	31	81	0.38	43	83	0.52	28	89	0.31
88	11	85	0.13	81	82	0.99	54	84	0.64	59	86	0.68
Averages................	20	66	0.35	35	68	0.52	27	73	0.38	33	77	0.43
Averages obtained from the same rabbits without pituitrin.....	70	82	0.79	98	82	1.11	108	81	1.24	120	85	1.44
The amount by which the average ratios obtained after pituitrin are less than the average ratios obtained without pituitrin......			−0.44			−0.59			−0.86			−1.01

DISCUSSION

We have shown that the subcutaneous injection of pituitrin is followed by a marked depression of the activity of the kidney in the excretion of urea.

There is no more ground for the supposition that this depressing action of pituitrin is the result of circulatory changes in the kidney than there is for ascribing the accelerating action of adrenalin to alterations in renal blood supply. What we know of the effect of these substances on the vessels of the kidney stands in direct opposition to any such hypothesis. Pituitrin given intravenously increases the volume of the kidney and causes diuresis (1). Adrenalin given intravenously decreases the volume of the kidney and stops the flow of urine (1). Outside the body pituitrin dilates the renal artery while adrenalin constricts it (2). If they have any such influence on the renal vessels when given subcutaneously one would expect the circumstances to counteract, rather than to cause the changes in kidney activity which we have shown they produce. But as a matter of fact there is no reason to suppose that pituitrin absorbed from the tissues has any vascular effects at all. Taukow (3) found that in rabbits, the subcutaneous injection of as much as 5 cc. of the extract we used did not alter the blood pressure. Yet one-hundredth part of this amount given in the same way will markedly reduce the urea excreting activity of the rabbit's kidneys. Here, as in the case of adrenalin, there seems reason to distinguish between the effect on the action of involuntary muscle and on the secretory action of the kidney, in the sense that very much smaller concentrations are more effective in the one case than in the other.

CONCLUSION

The subcutaneous injection of pituitrin (Parke, Davis & Co.) is followed in all effective amounts by a decrease in the urea excreting activity of the rabbit's kidney. The rate of urea excretion is slower than in animals not given pituitrin, although the blood urea concentration is higher.

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THE ACTIVATION OF MUSCLE CATALASE BY LIVER

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It is well known that thyroid feeding increases oxidations, and the results herein described are a sort of by-product of an attempt to ascertain if the thyroid could in any way be connected with catalase. Rat carcinoma was chosen for the reason that it is rich in catalase, and it was thought that there was a possibility of showing some connection between the thyroid and catalase that might account for the oxidation processes that must be going on where there is such a rapid cell division. In seeking for a control it was observed that the addition of small quantities of liver very markedly increased the activity of the catalase. The original problem was therefore dropped for the time being, and attention given to this phenomenon.

The liver and the leg muscles of the rat were used, as being at the two ends of the scale of catalytic activity; the liver being most active and the muscle least so, if we except the brain. The animals were killed and immediately perfused with a solution of sodium chloride of m/6 concentration and made up with tap water. A cannula was introduced into the aorta and the solution allowed to run until the fluid coming from the jugular vein was clear. Incisions into the liver showed it to be free from blood. The liver and muscles were then removed, passed through a meat grinder and the pulpy mass thus obtained was used in weighed quantities.

Commercial hydrogen peroxide was diluted with an equal volume of distilled water, making a 1.5 per cent solution of H_2O_2. It was used both neutralized and unneutralized. The oxygen was collected in a burette over water in the usual way, the amount given off in ten minutes being taken as the standard. As some of the work was done at noon and some at night, there was a variation of several degrees in the temperature and therefore the gas volumes are all reduced to 0° and 760 mm. Hg, the fractions being disregarded. Unless otherwise stated, 50 cc. of the peroxide solution were used.

The acceleration of the catalase activity of muscle by adding a small amount of liver is shown by the following:

1 gram of muscle liberated 24 cc. of oxygen.
1 gram of liver liberated 156 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of liver, gave 126 cc. of oxygen.
0.98 gram of muscle + 0.02 gram of liver, gave 58 cc. of oxygen.

The objection might be made that as the liver contains so much more catalase, we are dealing here simply with an increase of the active mass; 0.98 gram of muscle plus 0.02 gram of liver represents about 1.05 grams of muscle. This slight increase in amount could hardly cause an increase of 140 per cent in activity.

To make certain of this point, the following combination was made:

1 gram of muscle gave 50 cc. of oxygen.
1 gram of liver gave 244 cc. of oxygen.
0.5 gram of muscle + 0.1 gram of liver gave 250 cc. of oxygen.

Assuming the amount of catalase in the liver to be five times greater than in muscle, the above combination of muscle and liver represents 1 gram muscle; yet the amount of oxygen given off is that of the liver rather than of the muscle.

0.5 gram of muscle gave 23 cc. of oxygen.
0.1 gram of liver gave 41 cc. of oxygen.

The sum of these, 64 cc., is far below that for the same quantities when used in combination.

The following was then tried, assuming the liver to contain ten times as much catalase as the muscle:

0.5 gram of muscle + 0.02 gram of liver gave 210 cc. of oxygen.

In the earlier experiments other tissues of the same animal were used as controls. For instance, in experiment 7, rat,

1 gram of muscle gave 38 cc. of oxygen.
1 gram of liver gave 203 cc. of oxygen.
0.9 gram of muscle + 0.1 of liver gave 200 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of blood clot gave 158 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of thyroid gave 44 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of spleen gave 51 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of kidney gave 84 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of pancreas gave 32 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of testes gave 29 cc. of oxygen.

It will be seen that there is a small amount of acceleration with all but the pancreas and testes, where there seems to be a slight retardation. It should be pointed out that in the case of the spleen and kidney, they were not entirely free from blood. It is rather difficult, in perfusing the entire animal, to rid these organs of all blood. Aside from this it is quite evident that the liver has an accelerating effect far in excess of the other organs, and that the blood comes next in efficiency.

These experiments were done with acid H_2O_2. They were repeated with freshly neutralized H_2O_2 with the same results, the difference being in the magnitude of the figures. It was noticed, however, that whereas the liver acted much more energetically in the neutral peroxide, the muscle did not seem to be affected by the reaction; it acted as well in the acid as in the neutral peroxide.

These are the facts. The interpretation of these facts is not so clear. Some years ago Battelli and Stern (1) claimed to have obtained a substance, philocatalase, which had the property not only of antagonizing anticatalase but also of regenerating the catalase. They also describe an "activator" of the philocatalase. DeWaele and Vandevelde (2) throw doubt upon the existence of anticatalase, and therefore upon the existence of philocatalase and its activator. Loevenhart (3) observed an acceleration when pancreas and liver, and muscle and liver were combined, but he regarded it as an activation of the liver by the pancreas and muscle and thinks it due to a neutralization of the acid by some substance contained in these tissues. It has already been stated that in the present observations it was noted that liver was retarded by the acid, and that muscle acted equally well in acid or neutral peroxide, but when they were added together there was a more marked acceleration in the neutral peroxide. As an example,

0.5 gram of muscle + 0.02 gram of liver, acid H_2O_2, gave 170 cc. oxygen.
0.5 gram of muscle + 0.02 gram of liver, neutral H_2O_2 gave 240 cc. oxygen.

From these results it is clear that there is something in the liver catalase that does not exist in the muscle, or that liver catalase is different from muscle catalase.

In a preliminary communication read before the Pacific Coast Branch of the Society for Experimental Biology and Medicine at its January meeting, it was suggested tentatively that the liver secreted an activator of catalase, possibly in the nature of an internal secretion, and it might be well to point out the reasons for such a suggestion. In the first place, the blood is nearly as effective as the liver, and this one would expect if we are dealing with an internal secretion. Also the slight accelerating effect of practically all the organs could thus be accounted for, as well as the varying catalytic activity of the different tissues. Burge (4) describes an increase of catalase in muscle during activity, and this might be due as well to an increased amount of an accelerator carried to the muscle by the increased blood supply, as to an actual increase in the amount of catalase. So far as I know his estimations were all based upon the catalase activity of the muscle and not on the actual determination of the amount of catalase present. Finally, experiments now in progress with the catalase of liver obtained by the method of Battelli and Stern (5), seem to show that the accelerating property disappears in the preparation of the catalase. Enough work along this line has not been done, however, and it must be left for a future communication if the times, so sadly out of joint, will permit.

SUMMARY

1. In both acid and neutral hydrogen peroxide, the addition of a small amount of liver to muscle increases the catalytic activity of the mass.

2. Blood also has an accelerating effect on muscle catalase, nearly equal to that of liver.

3. It is suggested tentatively that this accelerating action may be due to an internal secretion, and the reasons for such a suggestion are given.

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MUSCULAR STRENGTH AND MUSCULAR SYMMETRY IN HUMAN BEINGS

I. In Children

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The observations on which this report is based were obtained in connection with a study of the causes and treatment of infantile paralysis, conducted under the auspices of the Vermont State Board of Health and financed through the generosity of an anonymous donor.

The series of papers of which this is the first represents an attempt to elucidate the factors which determine effective muscular strength in human beings, as displayed in maximal volitional efforts. The application by Weber and others (1) of the principle of absolute muscular force to human muscles gives us some idea of the relative strength of isolated human muscles in comparison with similar muscles of lower animals; the numerous investigations with the ergograph (2) throw much light on the conditions which influence muscular endurance; the literature of physical training contains many data relative to "strength tests" applied in gymnasia to athletes, and in a few cases for the purpose of aiding in the prescription of therapeutic exercise (3); but none of these has given rise to a generalization which will enable us to forecast for any individual his probable maximum strength and to analyze observed departures from expectation. Aside from the familiar fact that strength increases with exercise we have had hitherto practically no data on which to base such a generalization.

Effective muscular strength, by which is meant the power developed at the actual points where strength is ordinarily exerted, depends on a number of factors. The muscles work for the most part in groups, and through action on levers. The effective strength, for example, of the calf muscles in such an action as rising on the toes has a complex mechanical basis, in comparison with the absolute muscular force of the isolated gastrocnemius. Moreover, in exhibitions of voluntary muscle power the mechanism involved is a neuro-muscular one, in which the nervous part may have as much hand in determining the degree of activity as the purely muscular. Thus, so far as the actual use of the muscles is concerned, effective strength rather than intrinsic muscle power is of significance.

Source of the material. In connection with the development and use of a system of muscle-testing intended primarily as a feature of the after-care of infantile paralysis (4), a large series of observations of normal muscular strength was obtained. Part of the tests were made in the Orthopedic Department of the Children's Hospital, Boston. Most of the others were made at various points in Vermont.

The muscle test. Detailed descriptions of the system of muscle-testing are given elsewhere (4), (5). For the present purpose only the general features of the test will be described. The value obtained for each muscle-group is the "breaking strength." By this is meant the tension shown on a spring-balance at the instant the resistance of the contracted muscle is overcome by a pull in the opposite direction, exerted through the balance. Traction is afforded by a sling placed at a selected point on the part in which the muscle-group has its insertion. Standard positions for the sling and for the regions involved are used. These are so selected as to be easily located and to afford satisfactory mechanical conditions. Care is taken throughout that all purely physical factors, such as line of pull, shall be kept as nearly constant as possible. In this investigation the readings were taken in pounds and the attempt was made to read to the nearest half-pound. Tests on eleven muscle-groups on each arm and ten on each leg were used, a total of forty-two. These are enumerated in table 2. An additional arm group, the abductor of the thumb, was tested, but this is so weak, especially in young children, that the probable error on a scale read only to half-pounds is too great to justify its inclusion in a statistical study. This group is omitted, therefore, in all the data considered herewith.

Subjects. Tests available for this study were had on two hundred and forty individuals. These included one hundred and twenty-eight males and one hundred and twelve females. All ages were represented from four to eighteen. The age distribution is given in table 1. Since only the most advanced four-year old children could be reliably tested they are not listed separately, but are included under age 5. Many of the subjects (one hundred and sixty-eight) were patients on whom the diagnosis of infantile paralysis had been made. In all save seven the acute stage was a year or more in the past. The others were such as came to hand conveniently while tests were in progress. Many of them had localized abnormalities, such as club-foot or slight scoliosis, but in no case were the abnormalities of such a character as to vitiate the value of the readings taken. The group of subjects as a whole was fairly representative. There were a few cases from the slums of the city in which there was obvious under-nourishment. In most of the cases, however, there was no indication of malnutrition. A large percentage of the subjects were cripples in whom active exercise was a matter of difficulty. Although one would prefer for a study of this sort subjects who had not suffered from a disabling illness, the opportunity of obtaining a series of normal children even approximately as extensive as this seemed too remote to justify postponement of the present analysis. It may be said, furthermore, in favor of this group of subjects, that the less amount of activity would tend to diminish the occurrence of special exercise effects, leaving the group particularly representative of the conditions determining general muscular strength, independent of the effects of specific exercises. In this respect it was, perhaps, as satisfactory as would have been a group of strictly normal individuals.

TABLE 1

Subjects tested, grouped according to sex and age

AGE	MALES	FEMALES	TOTAL
5	16	12	28
6	11	8	19
7	20	17	37
8	16	16	32
9	14	17	31
10	6	11	17
11	13	4	17
12	6	4	10
13	3	6	9
14	1	2	3
15	6	1	7
16	6	5	11
17	6	2	8
18	4	7	11
Totals............	128	112	240

Musuclar symmetry. The expression of strength in this study is taken as the sum of the observed strengths of the muscle-groups listed in table 2. Inasmuch as so large a proportion of the subjects had some muscle-groups not of normal strength, and therefore not possible of inclusion, a method had to be devised at the outset by which allowance could be made for the omitted muscle-groups in stating the total strength of the individual. The method adopted was to determine for this series of subjects the average percentage distribution of strength among the various muscle-groups. As a first step in this procedure the subjects were classified by ages. Then the average strength of each muscle-group was determined for all the subjects of a given age. On the basis of these average figures the percentage distribution of the total strength among all the muscle-groups was determined for each age and the result tabulated. An interesting point brought out by study of this tabulation is confirmation, for children, of the fact established by Kellogg for adults (loc. cit., table 1) that there is much less difference between the right and left sides of the body than is generally supposed. In fact, the percentage differences between the two sides are neither great enough nor constant enough to involve serious error if the two sides of the body are assumed to be equally strong. In order to simplify the mathematical procedure this assumption was adopted. It was found, furthermore, that the percentage distributions of muscular strength for the various ages fall naturally into three series. The first of these includes the ages 5, 6 and 7; the second the ages from 8 to 12 inclusive; and the third the ages from 13 to 18 inclusive. For the practical purposes of this study the averages for the different muscle-groups for these age groupings were taken. These are set forth in table 2. In a later section, under the heading "Strength Distribution" the validity of table 2 is considered statistically. As a matter of interest, the actual average percentage distribution of strength for the two sides of the body and for the arms and legs, for the different age groups, is given in table 3.

The calculation of total strength.—The method of computing total strength when some muscle-groups are missing is as follows: The aggregate strength of the muscle-groups on which tests have been made is determined (designated A); from the figures of table 2 the percentage of the total strength represented by those muscle-groups is found (designated P); the calculation of the theoretical entire strength (designated T) is then made according to the formula $T = \frac{A}{P}$. As a check upon

TABLE 2

Average percentage distribution of strength among the muscles

MUSCLE-GROUP	AGE		
	5 to 7	8 to 12	13 to 18
Feet			
Plantar flexion	7.60	9.30	9.30
Dorsal flexion	3.35	3.10	3.20
Inversion	2.05	1.95	2.10
Eversion	1.95	1.85	2.00
Hips			
Adduction	1.60	1.50	1.55
Abduction	1.50	1.45	1.50
Extension	3.15	3.05	3.00
Flexion	3.35	3.20	3.10
Knees			
Extension	3.50	3.20	3.30
Flexion	1.80	1.70	1.70
Shoulders			
Pectoralis	2.20	2.10	2.10
Latissimus dorsi	1.45	1.50	1.45
Anterior deltoid	2.00	2.00	2.00
Posterior deltoid	1.45	1.45	1.50
Forearms			
Extension	1.95	1.75	1.60
Flexion	2.60	2.55	2.50
Wrists			
Extension	1.25	1.25	1.35
Flexion	2.25	2.15	1.90
Fingers			
Extension	0.70	0.70	0.70
Flexion	2.75	2.75	2.75
Thumbs			
Adduction	1.55	1.50	1.40

TABLE 3

Actual percentage distribution of strength

REGION	AGE		
	5 to 7	8 to 12	13 to 18
Left side	49.40	49.30	49.70
Right side	50.60	50.70	50.30
Arms	40.40	39.30	38.80
Legs	59.60	60.70	61.20

this method of computing total strength and also in an attempt to establish a means of obtaining an approximate measure of strength without actually having to carry through complete tests, the same method of calculation was applied, using only the extensors and flexors of the forearm and the extensors and flexors of the wrist as the basis for calculation. It appears that in a large proportion of individuals the strength calculations based only on the muscles of the forearm and wrist agree reasonably with the results of the complete tests. In the one hundred and twenty-six cases of this series in which this comparison was made, all ages and both sexes being represented, one hundred and seven (85 per cent) agree within 15 per cent, and one hundred and eighteen (94 per cent) agree within 20 per cent. The Pearson coefficient of correlation for this comparison is 0.91 ± 0.0168. It seems fair to conclude that strengths calculated from tests that are nearly complete are valid within the limits imposed by the inherent errors of the test itself.

After the strengths of all the individuals in each age group had been calculated averages were made for each sex separately at each age from 5 to 18 years. These averages are set down in table 4. As is at once apparent from scrutiny of the table, the values here given are not to be looked upon as satisfactory final estimates of the average strengths for the various ages of human beings. The number of cases in this series is too small, particularly at some ages, to afford a basis for such final estimates. As a preliminary toward the establishment of definite figures for the mean strength at each age they serve a purpose, however.

The relation of strength to weight. The next step was to determine whether these figures of average strength could be related with any other known data which vary with age. The obvious comparison is with weight, which varies with age in a definite manner and in which, moreover, the variations have been worked out carefully by Bowditch (6) and others. In table 4 the average strengths for the various ages are tabulated opposite the average weights of the same subjects. That there is a definite relationship between strength and weight is at once apparent when the columns in the table headed $\frac{\textit{Strength}}{\textit{weight}}$ are examined. The figures in these columns, when taken column by column, so as to divide the sexes, show an approximate constancy that is too striking to be accidental and that indicate a definite relationship between strength and weight. The column for the ratio of strength to weight in males shows considerable divergence at the two ends. If the figures for ages 5, 17 and 18 are omitted the average of the entire column is

20, the mean divergence from this average being slightly less than 4 per cent, and the maximum divergence 10 per cent. The figure 20 seems thus to represent fairly the ideal ratio of strength to weight in male children. The average for the column of ratios of strength to weight in females, omitting ages 15 and 17, at which there were insufficient data, is 18, with a mean divergence of 6.8 per cent, and a maximum divergence of 19 per cent. The figure 18 may be looked upon as the ideal ratio of strength to weight in female children. The coefficient of correlation between strength and weight is, for male children, 0.93 ± 0.009, and for female children, 0.86 ± 0.019.

TABLE 4

Ratio strength to weight; both sexes; ages 5 to 18 years

AGE	MALES			FEMALES			AVERAGE HEIGHT, BOTH SEXES
	Average weight	Average strength	$\frac{\text{Strength}}{\text{weight}}$	Average weight	Average strength	$\frac{\text{Strength}}{\text{weight}}$	
	lbs.	*lbs.*		*lbs.*	*lbs.*		*inches*
5	37	645	17.4	37.0	650	17.3	41
6	41	830	20.2	42.0	700	16.7	44
7	49	970	19.8	46.0	875	19.0	47
8	53	1050	19.8	58.0	1020	17.6	49
9	59	1260	21.0	55.0	1040	18.9	51
10	68	1380	20.2	62.5	1140	18.6	53
11	72	1490	20.7	79.5	1265	15.9	56
12	85	1600	18.8	104.0	1610	15.1	58
13	88	1750	19.9	92.0	1640	17.9	60
14	127	2300*	18.1	94.0	1710	18.2	62
15	102	1870	18.3	135.0	1760*	13.1	64
16	113	2380	21.1	114.0	2450	21.5	66
17	113	2680	23.2	83.5	1220†	14.6	67
18	155	3590	23.2	135.0	2500	19.1	67

* One case only.

† Two cases only, both severely affected by previous attacks of infantile paralysis.

The ratio for male children at age 5 is seen in the table to be 17.4. This figure is so closely approximate to the general ratio for female children as to suggest that at that age there is no sex difference in respect to strength. A point that may be worth making in this connection is that in this series of subjects the average weights at age 5 are the same for both males and females (see table 4). If one takes the ground that both weight and strength are infantile in type up to this age the suggestion follows that the female standard represents a continuation of the infantile ratio, while in the male there is a departure therefrom in the direction of a greater strength per unit of weight. It should be noted that the difference of ratio in favor of the male is not great, being only 10 per cent. The ratios for ages 17 and 18 in males are definitely higher than the average for the earlier ages. These higher ratios indicate that in adults there may be regularly higher ratios than in children, and that boys of 17 and 18 are attaining the adult condition in this regard.

TABLE 5

Comparison of actual strengths for the various ages with theoretical strengths computed by multiplying the Bowditch figures for weight by the ratios of strength to weight as established for the two sexes

AGE	MALES			FEMALES		
	Bowditch weight	Calculated strength	Actual strength	Bowditch weight	Calculated strength	Actual strength
5	41.1	740	645	39.7	710	650
6	45.2	905	830	43.3	780	700
7	49.1	980	970	47.5	855	875
8	53.9	1080	1050	52.0	935	1020
9	59.2	1180	1260	57.0	1030	1040
10	65.3	1310	1380	62.3	1120	1140
11	70.2	1400	1490	68.8	1240	1265
12	76.9	1540	1600	78.3	1410	1610
13	84.8	1700	1750	88.6	1600	1640
14	94.9	1900	2300*	98.4	1770	1710
15	107.1	2070	1870	106.1	1910	1760*
16	121.0	2420	2380	112.0	2020	2450
17				115.0	2070	1220†
18				115.2	2075	2500‡

* One case only.

† Two cases, both severely paralyzed.

‡ The average weight of these subjects was 135 lbs.; 20 lbs. more than the Bowditch figure.

As a further check upon the validity of the ratios of strength to weight here proposed, the theoretical strengths for each age in males and females were calculated by multiplying the mean weights given in the Bowditch table by the proposed ratios namely, 20 for males from 6 to 16 years of age and 18 for males of 5 years and all females. These theoretical strengths (see table 5) were then compared with the actual strengths previously found. If we omit from the comparison age 14 in males and ages 15, 17 and 18 in females, the first three because of insufficient data and the last because the 18 year old females of this series greatly outweighed the Bowditch figure for this age, the mean difference between the theoretical and the actual strength-averages is only 6.1 per cent. The coefficient of correlation between the two sets of averages is 0.977 ± 0.007. If all ages are included except age 17 in females, which obviously should under no conditions be counted, the mean difference becomes 7.1 per cent. This very close correspondence strengthens the view that in the ratios proposed we have a close approximation to what we may call the normal relationship between the strength of the selected series of muscle-groups on which this study is based and the body-weight.

The strength-weight ratios proposed above were determined on the basis of average strengths and average weights for each age. If they represent truly the relationship assumed for them they should apply to individuals as well as to averages, and they should hold good in those subjects whose weight does not correspond with the average for their age as well as in those whose weight agrees with age expectation. To test their individual application in these regards is the purpose of the next portion of this paper. Among the subjects tested were ninety-eight males and ninety-one females, one hundred and eighty-nine altogether, whose weights were determined at the time the tests were made. Unfortunately not all the cases examined could be weighed because in several of the towns in which tests were carried on it was not found possible to obtain the use of suitable scales. The average weights set down in table 4 were calculated from the subjects that were actually weighed, and the average strengths were calculated from all the subjects of the proper age, regardless of whether their weights were known or not. Thus the two sets of averages are not based on identical cases throughout. This makes it all the more desirable to check the assumed strength-weight ratios by applying them to individuals. A simple means of doing this is to determine the strength-weight ratio for each subject whose weight is known and then to find the average for each sex separately. When this was done the average for males was found to be 19.9 and for females 18. These ratios agree closely enough with those obtained above to serve as verification of them.

A well-known element in the acceptance of averages as truly representative is that the individual data on which they are based shall be shown to cluster around them within reasonable limits. It is not easy to decide for this set of data what are the proper limits; in other words, to tell how widely the strength of an individual may vary from the theoretical and he still be considered of "normal" strength. In view of the many factors that may influence strength and of the errors that are bound to inhere in a system of tests based on volition, it seems to me reasonable to allow a variation of 15 per cent on either side of the theoretical before considering the subject to be either below or above "normal." Of the one hundred and eighty-nine cases here under consideration one hundred and nineteen (63 per cent) were of "normal" strength according to the criterion just proposed. Thirty-five (18.5 per cent) were below "normal" and an equal number above "normal." Since the limits of "normality" are purely empirical, as they must be on so meager data, caution must be observed in drawing conclusions as to the factors which condition departures from "normality." It happens that in this series the individuals listed as "not normal" are equally divided between the sexes. This is true both of those that are of less than "normal" strength and of those that are stronger than the average. There is nothing to indicate a greater liability to divergence at some ages than at others although this series is not large enough to bring out such a tendency even though one were present. Scrutiny of the individual cases included under the captions "less than normal" on the one hand and "more than normal" on the other suggests that many of them were actually weaker or stronger than the average for their ages, so that their presence in the series helps to account for the relatively large proportion of the total which falls outside the "normal" limits. Thus half of those in the list of "weaker than normal" were infantile paralysis cases in which the test of strength was made approximately a year after the onset of the disease. The percentage of similarly recent onsets among the "normally strong" cases is less than half as large. Almost without exception the children in this group presented an appearance of delicacy which would naturally be associated in the mind of an observer with less than normal muscular strength. The group of "more than average strength" consisted of children who were, to the most casual observation, of unusual ruggedness and vigor. More than half of them, nineteen out of thirty-five, were undersized. That is, they were both shorter and lighter in weight than the average for their ages. One would be inclined to expect to find a tendency toward a high strength-weight ratio among those who are small for their ages, provided the small size does not involve a serious deficiency in amount of muscular tissue. On the whole, the departures from "normality" in this series do not seem to be more extensive than may properly be anticipated. The further condition to be fulfilled by the strength-weight ratios, as stated above, is that they shall hold for individuals whose weight falls outside the expectation for their age as well as for those whose weight accords with age-expectation. Among the one hundred and eighty-nine cases making up the series now under examination there were seventy-seven whose weight varied from the average for their age by five pounds or more. Forty-five of these were underweight and thirty-two overweight. Twenty-three of the overweight (72 per cent) were included among the "normally strong." Seven were weaker than "normal" and two stronger Twenty-one of the underweight, slightly less than half, were of "normal" strength. Nineteen, as stated above, were stronger than "normal" and five weaker. Except for the probability, already mentioned, that undersized persons are likely to have a high strength-weight ratio, it is seen, thus, that the adopted ratios hold for individuals who are not of average weight for their age.

The relation of strength to height. Another factor which varies in a fixed manner from year to year of age and which, therefore, may properly be compared with strength, is height. At first glance one would be inclined to suppose that effective strength would vary inversely with height so long as other factors remained constant. The greater length of arms and legs would appear to diminish the effective leverage of many muscle-groups. As a matter of fact this series of subjects gave no indication that undue height is accompanied by relatively less strength. Of twenty-three individuals who were decidedly taller than the average for their weight only two showed less than "normal" strength. Five, on the other hand, were stronger than "normal." In general the relationship of strength to height seems to be that which should follow from the ratio of strength to weight as stated above and the relationship of height to weight as determined by the principle that the mass varies as the cube of the length. Since this principle applies in human beings to the ratio of weight to height and since the relationship of strength to weight is direct, it follows that the strength should vary as the cube of the height. This it does approximately, but the presentation of a curve in demonstration of the fact seems unnecessary inasmuch as all the essential data have been already presented in tables above. (See table 4 for average heights.)

Strength-distribution. In addition to affording evidence that the strength tends to bear a fixed ratio to weight and a definite relationship to the cube of the height, the data here under examination furnish a means of determining the average distribution of the strength among the muscle groups, in other words the "muscular symmetry." In an earlier section, as an aid to the calculation of total strength when some muscle-groups were not available for testing, the table of ideal strength-distribution is set down (table 2). The subject is reopened here in order to emphasize the possible significance of data of this kind, especially in connection with the interpretation of specific exercise effects or of limitations of activity due to habits or clothing (7). Obviously the possession of ideal standards of strength-distribution is the first requisite toward such interpretations. The particular standards here presented are based upon averages from a rather limited series of cases. For that reason they are subject to some modification as more data are accumulated. Their validity as standards must be established, furthermore, by the demonstration that they are truly representative, that the deviations of individual cases from the averages proposed as standards are not unduly wide. In the table of strength-distributions (table 2) standards for three age groups are submitted instead of standards for each age. Moreover, the same figures are proposed for given muscle-groups on the right and left sides of the body, notwithstanding that there is a slight general preponderance of strength on the right side. Although in making these changes certain errors are introduced, the practical advantage is so great, since the number of figures in the table is reduced from 588, the number when all ages and both sides of the body are included, to 63, the present number, that the errors are permissible provided they are not too great. The extent of error is indicated by a direct comparison of the substituted figures with those they are designed to replace. This comparison shows that 83 per cent of the 588 figures in the large table agree with the substituted figures within 10 per cent; all but 45 of the 588, or 93 per cent, agree within 15 per cent; and the average deviation of the entire series is only 6.2 per cent. Furthermore, more than half of the wide deviations (29 out of 45) are in the ages 13, 14, 15 and 17, in which, as table 1 shows, there were the fewest subjects and in which also the additional factor of differences in strength-distribution on account of sex might be expected to show if such differences exist.

The real test of the validity of the figures proposed as standards of strength-distribution must come from a comparison with them of the strength-distribution in individuals. This comparison has been made in all of the two hundred and forty cases making up this series. The method used was to determine for each subject the percentage of his calculated total strength represented by each of his normal muscle-groups, and then to find the percentage deviation of each group from the standard as given in table 2. The mean deviation from standard of all the normal muscles in any individual gives an expression of his symmetry. Obviously, if his strength-distribution corresponds exactly with that given in table 2 for his age-group his mean deviation will be zero; the figure for mean deviation will become larger and larger as various muscle-groups differ more and more widely from the standard.

TABLE 6

Mean deviations from ideal symmetry. The numbers in the different columns stand for the number of cases whose mean deviations fell within the limits indicated at the head of the columns

AGE	MEAN DEVIATIONS NOT MORE THAN 12.5 PER CENT	MEAN DEVIATIONS BETWEEN 12.6 AND 17.5 PER CENT	MEAN DEVIATIONS BETWEEN 17.6 AND 22.5 PER CENT	MEAN DEVIATIONS MORE THAN 22.6 PER CENT
5	8	7	7	6
6	2	11	6	
7	8	12	14	3
8	5	15	10	2
9	6	14	8	3
10	6	5	6	
11	3	6	7	1
12	5	4	1	
13	4	3	2	
14		1	2	
15	1	2	3	1
16	2	6	2	1
17		4	2	2
18	3	4	2	2
Totals........	53	94	72	21

Average deviation from ideal symmetry of entire series of 240 cases = 16.7 per cent.

For convenience of reference the observed mean deviations for the subjects of this study are tabulated in table 6. The range of deviations in the different columns was selected arbitrarily but with the idea of grouping the exceptionally symmetrical cases in one column, the fairly symmetrical in another, the somewhat unsymmetrical in a third and the definitely non-symmetrical in a fourth. The physical appearance of the subjects, as suggesting symmetry or non-symmetry, had something to do with the establishment of the limits of the different columns. Those whose mean deviations did not exceed 12.5 per cent were to the most casual inspection exceptionally symmetrical in bodily configuration. Those that fell in the second group, with mean deviations not exceeding 17.5 per cent, presented the physical appearance of symmetry, probably not distinguishable from the members of the first group except, perhaps, on the basis of actual measurements. The third group, consisting of subjects whose mean deviations fell between 17.6 per cent and 22.5 per cent, included many who were definitely recognizable as likely to be somewhat unsymmetrical; thus in this group were a number who were conspicuously strong for their age and size; others were as evidently weaker than normal; still others showed perceptible disproportion between arms and legs. All these conditions might tend toward diminished symmetry. The members of the fourth group, with few exceptions, were cases that might be picked out by any careful observer as unsymmetrical, except for three very young children, included in the list of 5-year-old cases, but actually only 4 years of age. It is probable that the tests on these very young children were less reliable than those of the older subjects. One 11-year-old girl and two boys, 16 and 18 years old respectively, gave tests which brought them into this group, without there being any obvious physical departure from symmetry. All the others of the fourth, or non-symmetrical group, were readily recognizable from mere inspection as belonging to it.

Included in columns 1 and 2 of table 6 are one hundred and forty-seven cases, reckoned in this classification as fairly or exceptionally symmetrical. These make up 61 per cent of the entire series of two hundred and forty cases. Included among the remaining 39 per cent or ninety-three cases, are eight that exceed the limit of column 2 by so narrow a margin that a change of 10 per cent in the right direction in the single muscle-group furthest removed from ideal symmetry would bring them into column 2, and at least thirty-five cases that for one reason or another would be expected to show considerable departure from ideal symmetry. The remaining fifty cases constitute a group of unexplained deviation from symmetry which is probably no larger than may be expected to appear in such a series as this. When we recall the nature of the test together with the readiness with which individual muscle-groups may be developed by special exercises and the likelihood that in any group of two hundred and forty children a number would be found who had been accustomed to using particular muscle-groups to a greater degree than common, the percentage of satisfactorily symmetrical cases seems ample.

An additional check upon the validity of the proposed standards of strength-distribution is afforded by the study of individual muscle-group percentage distributions as distinct from the averaged records of all the tests upon single subjects. Since a mean deviation of 17.5 per cent was adopted as the upper limit of reasonable symmetry in the subject, a slightly higher deviation, 20 per cent, may be permitted in the individual muscle-group. Of the entire number of muscle-groups tested 70 per cent agreed with expectation within 20 per cent, the remaining 30 per cent diverging from expectation more widely than 20 per cent. In the case of individual muscle-groups "expectation" consists of the percentage figure for the muscle-group in question as set down in table 2. An agreement within satisfactory limits of 70 per cent of all the muscle-groups tested is probably as good as can be hoped for, considering the nature of the tests. So far as the material at hand is concerned there seems sufficient justification for the adoption of the average strength-distributions of table 2 as standards of symmetry in children.

DISCUSSION

The early part of this paper is devoted to an account of observations which show that in children the strength tends to bear a fixed relation to body-weight. In placing this interpretation upon the observations no violence is done to prevailing ideas. It is a commonplace that the strength of individual muscle-groups is determined largely by the use to which those groups are put. In cases where specific exercise effects are not present, which are precisely the sort of cases to which this discussion is intended to apply, the one element of muscle-use which is constantly operative and which enters as a factor in all muscular movements, is weight, either of the entire body or of the part of the body in which a particular muscle-group has insertion. The strength-weight ratios suggested by these observations are, of course, applicable only in connection with this special system of muscle-testing and they are subject to minor modification when larger series of tests are available, but the principle here advocated, that in the absence of specific exercise effects the body-weight is the determining factor of strength, seems worthy of serious consideration. If this principle can be definitely established we have a generalization on which to base expectations of strength in individuals, and a point of departure from which to analyze the effects on strength of given amounts or kinds of exercise.

Similarly, as brought out above (p. 77), information as to the ideal distribution of strength among the muscle-groups may enable us to interpret and perhaps to regulate departures from the ideal due to special exercises or to particular habits. Standards of muscular symmetry may come to have a value comparable to that of standards of anatomical symmetry.

As a contribution toward the establishment of quantitative standards of physiological activities these observations are submitted.

SUMMARY

1. Strength tests by means of a spring-balance method were made on two hundred and forty children between the ages of 5 and 18 years.
2. The average percentage distribution of the strength among the muscles of the body is determined for each age. It is found that the percentage distribution of strength among the muscles can be stated for three age-groups, 5 to 7, 8 to 12, and 13 to 18 without introducing serious error. These percentage distributions (table 2) are proposed as standards of muscular symmetry in children.
3. The previous finding of Kellogg that there is relatively little difference in strength between the two sides of the body is confirmed.
4. Data are presented to show that calculations of entire strength based upon tests of only part of the muscle-groups in the body are valid within a reasonable margin of error.
5. The average strength for each age is found. It is somewhat less in females than in males.
6. The ratio of the average strength for any age to the average weight for the same age, keeping the sexes separate, is approximately constant. The value of this constant for males is 20 and for females 18. For males of 5 years or less the ratio is the same as for female children of all ages. Males above 16 years have a higher ratio than 20.
7. Application to individuals, regardless of their ages, of the above constants, shows that the ratios apply within a reasonable limit to 63 per cent of the cases examined.
8. The relation of strength to height is that which should follow from the principle that the mass varies as the cube of a linear dimension and from the demonstration that the strength varies directly as

the weight. There is no evidence that undue height tends to reduce the effective strength.

9. The fixed ratio of strength to weight is interpreted as signifying that the effective strength, as manifested in volitional efforts, depends, in the absence of specific exercise effects, upon the constantly operative factor of weight, either of the entire body or of the part moved by a particular muscle-group.

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THE REGULATION OF RENAL ACTIVITY

VI. The Effect of Adrenalin and Pituitrin on the Action of the Kidney under Strain[1]

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By the term strain is meant the condition imposed on the kidney when it is called on to increase its rate of work because of an increase in the concentration of urinary constituents in the blood. In the case of urea this condition may be induced at will by the administration of preformed urea. The following table shows how the rabbit's kidney reacts to the strain resulting from the introduction into the stomach of 5 grams of urea.

TABLE 1

Effect of strain on the urea excreting activity of the kidney

PERIOD	WITHOUT STRAIN (Averages of 35 experiments on 27 rabbits given no urea)			WITH STRAIN (Averages of 143 experiments on 51 rabbits given 5 grams urea)		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	62	70	0.79	232	133	1.82
II	94	70	1.25	417	220	1.98
III	106	73	1.38	481	236	2.08
IV	111	75	1.56	488	233	2.11

Both of the groups of animals compared in the above table were under exactly the same experimental conditions except that the rabbits in the larger group were given 5 grams of urea dissolved in 25 cc.

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[1] A note on the effect of adrenalin and of pituitrin on the urea excreting function of the kidney under strain was published in the Proc. Soc. Exper. Biol. and Med., 1916, xlv, 49.

of water at the beginning of the experiment. The manner in which the kidney responded to the strain thus induced is shown in the rates of urea excretion for these periods. There is not only a marked increase in the amounts of urea eliminated, but this increase is relatively greater than the increase in the blood concentration, for while the urea in the blood is not much more than three times greater, the urea in the urine is well over four times larger in amount than in animals not given urea. Strain, therefore, is a condition under which the urea excreting activity of the kidney is not only absolutely but also relatively increased. The effort put forth by the kidney is more than sufficient to meet the increased demand for work. This state of renal hyperactivity is expressed in the greater magnitude of the ratio between the urea content of the urine and of the blood after urea administration.

It has been shown that the unknown factors in the regulation of renal activity whose nature we are investigating reveal their existence through the variations they induce in the rate of urea excretion, variations which cannot be accounted for as a result of changes in blood urea concentration. And it will be remembered that these variations become less marked the greater the strain on the kidney. For when the relative incidence of unexplained fluctuations in rate above and below the curve of the average rate was plotted on a scale according to the blood urea concentrations at which the rates were measured, a curve resulted which indicated that the variability of the rate was high at low blood concentrations but decreased as the blood urea concentration increased (1).

It has further been shown that at low blood concentrations all degrees of those variations in excess of the average rate, which at times and irregularly are observed under our standard conditions may be almost constantly and at will induced by the subcutaneous administrations of appropriate amounts of adrenalin (2). Similarly the variations below the curve of the average rate which appear spontaneously and occasionally in any series of observations may be duplicated experimentally by the injection of pituitrin (3).

It therefore becomes of interest to determine whether adrenalin and pituitrin have the further resemblance to the unknown factors of producing relatively smaller variations above and below the curve of the average rate of urea excretion when the kidney is under the condition we have defined as strain.

The effect of adrenalin and of pituitrin on the kidney subjected to strain is shown in tables 2 and 3. They give the averages of groups of rabbits without and with adrenalin or pituitrin. Five grams of urea dissolved in 25 cc. of water were given to each animal at the commencement of the experiment.

It is evident that the effect of adrenalin and of pituitrin remains qualitatively the same under strain as under conditions where there is no strain. The ratio is still increased by adrenalin and decreased by pituitrin. The difference between the levels of blood urea concentration in the control as compared with the adrenalin and pituitrin experiments is probably due to a retarding effect of both these substances on the rate of absorption of the administered urea from the gastro-intestinal tract, yet even here the divergent effect on the kidney can be traced, for while the blood concentration tends to fall toward the end of the experiment in the adrenalin animals, it continues to rise progressively when pituitrin is given.

TABLE 2

Effect of adrenalin on the kidney under strain

PERIOD	WITHOUT ADRENALIN (Averages of 24 experiments on a group of 9 rabbits given 5 grams urea)			WITH 0.5 CC. ADRENALIN (Averages of 14 experiments on the same group of 9 rabbits given 5 grams urea)		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	195	131	1.68	273	94	2.96
II	468	232	1.98	509	171	2.96
III	481	249	1.99	506	174	2.95
IV	461	240	2.01	457	165	2.78

TABLE 3

Effect of pituitrin on the kidney under strain

PERIOD	WITHOUT PITUITRIN (Averages of 16 experiments on a group of 8 rabbits given 5 grams urea)			WITH 0.25 CC. PITUITRIN (Averages of 12 experiments on the same group of 8 rabbits given 5 grams of urea)		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	224	126	1.64	175	100	1.72
II	482	228	2.08	267	186	1.47
III	490	242	2.04	276	208	1.34
IV	496	229	2.18	271	221	1.27

The point with which we are immediately concerned, however, is to determine whether the augmenting action of adrenalin and the depressing action of pituitrin is quantitatively different in degree under these conditions of strain as compared with the degree of their action under conditions which impose no extra work on the kidney. But the percentage of change in rates or ratios induced by adrenalin or pituitrin cannot be calculated from the averages of the control experiments because of the difference in the levels of blood urea concentration to which we have referred. These differences make the changes less than those which would have resulted had an equality of blood concentration been attained. It is, therefore, necessary to compare either the rates or the ratios after adrenalin or pituitrin with the general average rate or ratio obtained at the same blood urea concentration under the standard control conditions. But this also would be a faulty method. For the average rate and ratio curves we have given (4) are composites of rates and of ratios during each of the four periods of the experiment. But we have shown that the rates and ratios increase progressively during each successive period, even though the blood urea concentration remains the same (1). The curve of the average is, therefore, different for each period. We had, however, determined the curve of the average rate of urea excretion for each period separately and we have accordingly taken these curves as a more correct standard for comparison

TABLE 4

Comparison of the percentage deviations from the average rate of urea excretion produced by adrenalin when acting on a kidney without strain and when acting on a kidney with strain

PERIOD	ADRENALIN 0.5 CC. WITHOUT STRAIN (Averages of 13 experiments on a group of 13 rabbits)					ADRENALIN 0.5 CC. WITH STRAIN Averages of 15 experiments on a group of 9 rabbits)				
	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after adrenalin	Deviation from average rate	Percentage deviation	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after adrenalin	Deviation from average rate	Percentage deviation
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
I	42	30	54	+24	+80	94	160	273	+113	+71
II	48	45	82	+37	+82	171	332	509	+177	+53
III	50	58	104	+46	+79	174	380	506	+126	+33
IV	52	63	103	+40	+64	165	375	457	+ 82	+22

than the general curve we gave in our first paper. In tables 4 and 5 the percentage deviation produced by adrenalin and by pituitrin from the curve of the average rate for each period at the levels of blood urea concentration found when the kidney was not under strain, has been compared with the percentage deviation produced by the same amounts of adrenalin and of pituitrin from the curve of the average rate for each period at the levels of blood urea concentration found in the above experiments after the administration of urea.

TABLE 5

Comparison of the percentage deviations from the average rate of urea excretion produced by pituitrin when acting on a kidney without strain and when acting on a kidney with strain

PERIOD	PITUITRIN 0.25 cc. WITHOUT STRAIN (Averages of 9 experiments on a group of 9 rabbits)					PITUITRIN 0.25 cc. WITH STRAIN (Averages of 12 experiments on a group of 8 rabbits)				
	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after pituitrin	Deviation from average rate	Percentage deviation	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after pituitrin	Deviation from average rate	Percentage deviation
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
I	66	65	20	−45	−69	100	175	175	− 0	− 0
II	68	75	35	−36	−48	186	365	267	− 98	−27
III	73	105	27	−47	−45	208	465	276	−189	−41
IV	77	105	33	−44	−42	221	502	271	−231	−46

These figures show that strain considerably reduces the degree of deviation from the average. With low blood urea concentrations the rate of urea excretion is 76 per cent higher with adrenalin when the average deviation for all four periods is taken. Under strain the increase is only 45 per cent. With pituitrin a decrease of 51 per cent without strain is reduced to a decrease of 29 per cent under strain.

Adrenalin and pituitrin, therefore, resemble the unknown factors concerned in the regulation of renal activity, not only in producing deviations both above and below the plane of activity usually found at any given blood urea concentration but also in producing a lesser degree of deviation when the kidney is under strain.

CONCLUSION

At high blood urea concentrations the degree of change in the urea excreting activity of the kidney produced by adrenalin and by pituitrin is less than at low blood urea concentrations.

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THE RELATION OF THE ADRENALS TO PIQÛRE HYPERGLYCEMIA AND TO THE GLYCOGEN CONTENT OF THE LIVER

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PART I. THE RELATION OF THE ADRENALS TO PIQÛRE HYPERGLYCEMIA

We have recently (1) studied the question whether the epinephrin secretion of the adrenals is indispensable for the production of certain experimental hyperglycemias. The majority of previous investigations have suffered from the defect that they were carried out, if not on practically moribund animals, at least on animals still under the effects of a serious operation. This undoubtedly is the chief reason for the astonishing lack of uniformity in the results. Working with animals (cats) in which the epinephrin secretion was abolished or reduced to an insignificant fraction of the normal by removal of one adrenal and section of the nerves of the other (an operation which does not preclude the continued life of the animal in good health), we were able to show that two forms of experimental hyperglycemia—that produced by ether and that produced by asphyxia—are as readily obtained in the absence of epinephrin secretion as when the adrenals have not been interfered with. We purposely reserved the question of the relation of the adrenals to piqûre hyperglycemia for further investigation since it is the form which is commonly supposed to be more closely associated than any other with the activity of the adrenal medulla. In his earlier work Kahn (2) was unable to find evidence of increased epinephrin liberation following piqûre. His later statement (3) that the piqûre causes an augmented epinephrin liberation, even if it were well founded, would by no means settle the question. For his observations furnish no evidence that the quantity liberated is at all comparable to the quantity required to produce the typical adrenalin hyperglycemia and glycosuria when adrenalin is artificially injected. But in fact Kahn's experiments do not show that the output of epinephrin is at all influenced by the sugar puncture. Deductions from the relative depth of the coloration produced by chromium salts in the adrenal medulla have little or no quantitative value. And such estimations as he made by the Läwen method are vitiated by the fact that he took no account of changes in the rate of blood flow through the adrenals in the period for which the animal was allowed to survive following the piqûre. An increase in epinephrin concentration in the adrenal vein blood shown by the perfused frog preparation could not be interpreted as an increase in the output of epinephrin per unit of time unless it were known that the blood flow through the adrenals had not been proportionately diminished after the puncture, as compared with the flow during collection of the comparison samples before the puncture. Kahn did not even collect pure adrenal vein blood, but drew off blood from the inferior cava. The difference in vasoconstricting power of different samples of serum or defibrinated blood is also a factor which detracts greatly from the value of such estimations on blood-vessel preparations.

It has not been sufficiently recognized by some of the other investigators who have published experiments purporting to show an increased rate of liberation due to this or that factor that certain indispensable conditions must be fulfilled if the comparative tests are to have any quantitative value. When blood from the adrenals is withdrawn and then tested on such objects as rabbit intestine and uterus segments, it is practically always the concentration of epinephrin in the liquid which is estimated, since the concentration of adrenalin added to an indifferent specimen of the same blood which will give an effect equal to that of the adrenal specimen is determined. The condition of the segment in all observations which are to be compared must of course be approximately the same, as we have repeatedly pointed out in other papers. The concentration as thus estimated gives no information as to the rate of liberation of epinephrin per unit of time unless the mean rate of blood flow through the adrenals during collection of the given specimen is known.

When deductions are made in regard to the rate of liberation of epinephrin from experiments on test objects *in situ* it is of course just as necessary to see that in any observations compared the test object is in the same condition as regards reactivity, the rate of the blood flow through it, etc. For example, von Anrep (4) states that if the nerves of a hind limb or kidney be cut, these denervated parts respond to stimulation of afferent nerves (central end of sciatic) by a vasoconstriction if the adrenals have not been interfered with, but only show the initial passive dilatation due to the rise of blood pressure if the adrenals have been extirpated. He draws the conclusion that stimulation of the sciatic reflexly increases the rate of liberation of epinephrin. But granting that the characteristic vasoconstriction can only be elicited with intact adrenals (and von Anrep's careful work seems to leave little doubt that this is a fact) this does not at all prove that during stimulation of the nerve more epinephrin is being poured into the blood per unit of time than without stimulation, for during stimulation of the sciatic the condition of the test object is greatly altered. The rise of blood pressure must necessarily increase the rate of blood flow through the denervated region. Usually the increase will be very considerable. With the adrenals intact and steadily discharging epinephrin at the normal rate, this means that the amount of epinephrin passing per unit of time through the vascular tract in question is abruptly and markedly augmented. If such denervated areas are as sensitive to epinephrin as is claimed, they may be expected to respond to this increase in the amount of epinephrin traversing them, even if no change whatever has taken place in the rate of its liberation from the adrenals. This reaction, accordingly, is not at all out of harmony with our observations made by a more direct method; namely, collection of adrenal vein blood during and without afferent nerve stimulation, and testing of the blood specimens on rabbit intestine and uterus segments. If afferent stimulation really causes such a great increase in the output of epinephrin as some authors believe, we ought to have been able to capture some of this epinephrin-rich blood coming from the adrenals, but we found no difference in the output in samples collected in equal times with and without afferent nerve stimulation (5). It is obvious that if the phenomenon studied by von Anrep is due to passage through the denervated region of an increased amount of blood with the ordinary content of epinephrin, it will necessarily be abolished by excision of the adrenal or by clipping of the adrenal veins, just as if it were due to a greatly augmented output of epinephrin produced reflexly. All that can be deduced from the fact that after elimination of the adrenals the vasoconstriction in the denervated part is absent, is that epinephrin was being given off from the adrenals, a fact which is undisputed.

We have shown (6) that as a matter of fact an increase in the amount of blood supplied to a test object *in situ* (denervated eye) without change in the concentration of the epinephrin, is associated with an increased epinephrin reaction, or with the appearance of a positive reaction where none was elicited with the previous rate of blood flow. For example, if a strength and duration of stimulation of the peripheral end of one or of both splanchnics be sought which will just fail to cause dilatation of the pupil of the denervated eye, a definite dilatation will in general be obtained when certain alternative arterial paths are occluded at the proper time, so as to allow more of the epinephrin-containing blood to pass to that eye. The concentration of epinephrin in the blood will, of course, not be affected. A precisely similar result is obtained with artificially introduced adrenalin. Here it would be obviously absurd to say that the pupil reaction had been elicited because the rate of liberation of epinephrin from the adrenals or the rate of injection of adrenalin had been increased by clamping alternative paths at a time when the liberation or injection had been already completed.

Another, perhaps less important, factor may be involved in von Anrep's experiment, which likewise he has not taken into account. The reflex vasoconstriction associated with stimulation of the sciatic may be expected to cause, for a short time at least,—and this is all that concerns us,—a slackening in the flow through the splanchnic area and therefore a diminution in the quantity of blood coming to the inferior cava and mingling with the adrenal blood.[1] The epinephrin secretion proceeding unchanged at the normal rate, the concentration of epinephrin in the blood entering and leaving the heart will thus be increased, so that not only will the denervated region receive a much augmented quantity of blood, but the concentration of epinephrin in this blood may be greater than before the nerve stimulation without any increase having taken place in the output of epinephrin per unit of time.

Von Anrep himself performed an experiment, which we believe proves conclusively that the effects observed by him are not due to augmented epinephrin secretion but to a redistribution of the blood containing epinephrin given off at the previous steady rate. He says:

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[1] The observations of Edwards (7) on the compensatory increase of blood flow through the head and limbs during stimulation of the splanchnic are not out of harmony with the supposition that stimulation of the central end of the sciatic may cause an increase of concentration of epinephrin in the blood of the inferior cava in the manner suggested without any change having occurred in the rate of liberation of epinephrin from the adrenals. For we do not know that a similar compensation occurs, or that it occurs as promptly with stimulation of the central end of a peripheral nerve trunk, which may cause vasoconstriction in the head and limbs also, as when the peripheral end of a splanchnic is stimulated.

> It is important to note that if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of the denervated limb. Only after the other splanchnic nerve is cut does this constriction disappear and the limb react passively to the changes of blood pressure.

His interpretation of this result is that

> this is due to the fact that stimulation, even of the peripheral end (of the splanchnic), excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve.

There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal, and we have good evidence against it. The true explanation, we believe, is that the liberation of epinephrin is proceeding steadily at a substantial rate from the adrenal with intact splanchnic and blood containing a concentration of epinephrin corresponding to this discharge is passing steadily through the denervated region as through the rest of the vascular system. Whatever influence it is exerting cannot of course be revealed till some change occurs in the concentration or in the amount passing through per unit of time. When the splanchnic on the side of the extirpated adrenal is stimulated, there is no increase in the rate of liberation of epinephrin from the remaining adrenal but the vasoconstriction in the splanchnic area causes a rise of arterial pressure which is straightway reflected in an increased flow of the epinephrin-containing blood through the denervated area. This area naturally responds, granting that such vascular regions are as sensitive to epinephrin as von Anrep assumes, by a vasoconstriction to the larger amount of epinephrin offered to it.

The similar reactions elicited by asphyxia, and obtained not at all or in smaller degree after elimination of the adrenals, in like manner afford no evidence in favor of a greatly augmented output of epinephrin, certainly no evidence which can be set up against such direct tests as we have made with the actual adrenal vein blood (10). In the case of asphyxia an additional and a formidable complication is introduced in such indirect experiments as those of von Anrep by the fact that asphyxia may be expected to alter the reactivity of the test object to epinephrin. Unless this factor is controlled, it is impossible to say that an increased reaction is due to an augmented liberation of epinephrin and not to an increased sensitiveness of the test object to such amounts as were already present.

When we have used as test objects organs or tissues *in situ* we have endeavored to render the observations to be compared really comparable by collecting the blood to be tested in the cava pocket and only releasing it when the test object was again approximately under the original conditions. For instance, in determining whether stimulation of afferent nerves caused any change in the rate of liberation of epinephrin, we collected blood in the pocket for a given time, then released it and noted the effect on the eye and blood pressure. Then blood was collected for the same length of time during nerve stimulation. In many of our experiments the splanchnic area and hind limbs were purposely tied off, so that the reflex rise of blood pressure was usually negligible. But where the nerve stimulation produced any considerable rise of blood pressure the stimulation was stopped some time before the opening of the pocket so that the tissues of the eye-ball and the blood vessels might, as far as practicable, have the opportunity of reverting to the same conditions as regards rate of blood flow, etc., as were present in the comparison observation. Only after this interval was the epinephrin-containing blood in the pocket released. In our observations on asphyxia a longer interval was allowed to permit the blood pressure to return to normal, the respiratory movements to diminish and the asphyxial products, to some extent at least, to disappear. We never supposed that it was permissible to use in one observation an asphyxiated test object and in the comparison observation the same object with unobstructed respiration; or to assume that if there was any difference in the reactions it must be due to a difference in the rate of output of epinephrin, the condition of the test object itself being of no moment.

Certain reactions (especially acceleration) of the heart, when isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, have also been supposed to prove that the rate of output of epinephrin from the adrenals is greatly increased reflexly by stimulation of afferent nerves. It is not difficult, however, to see that the results of the study of these reactions by von Anrep and others must be interpreted precisely in the same way as the results on the denervated vascular areas, and yield no better evidence of reflexly augmented epinephrin secretion than the vascular reactions do.

Thus von Anrep (8), starting with the demonstration that stimulation of the peripheral end of the cut splanchnics is followed by the characteristic heart reactions if the adrenals are present, but not if they have been removed, is led to the generalization that

> every rise of blood pressure brought about by the agency of the nervous system involves the coöperation of the chemical mechanism represented by the suprarenal glands.

And it is clear that whether the rise of pressure be brought about by direct stimulation of the efferent splanchnic fibers, by asphyxia or by stimulaton of afferent nerves, he had no other conception of this coöperation than that the nervous action on the cardiovascular system is accompanied by a nervous action on the adrenals which causes an increased outpouring of epinephrin.

Nobody doubts that when the peripheral end of the cut splanchnic is stimulated the liberation of epinephrin from the corresponding adrenal, which has been reduced by the nerve section from the normal spontaneous rate to zero, or at least greatly diminished, is augmented. Von Anrep is therefore entitled to conclude that in this case a part of the characteristic vascular and cardiac reactions is due to the abrupt increase in the epinephrin content of the blood. But it is one thing to use the well established fact that stimulation of the peripheral end of the splanchnic increases the output of epinephrin to explain a reaction of the denervated heart which can be shown to depend upon the secretion of epinephrin by the adrenals, and quite another thing to deduce from the occurrence of these reactions when afferent nerves are stimulated the conclusion that they must be due to an *augmented* output of epinephrin, merely because they cannot be obtained in the absence of the adrenals.[2] This conclusion could only be accepted if it were shown that the increase in the rate of blood flow through the coronary circulation associated with the rise of blood pressure is inadequate to produce the reactions. The increased blood flow must necessarily be accompanied by an increased supply of epinephrin to the heart vessels per unit of time, even if no increase has occurred in the rate of liberation of epinephrin from the adrenals, and the denervated heart, an exceedingly delicate test object for epinephrin, according to von Anrep, may be expected to respond just as if the epinephrin output had been increased without any material change in the rate of flow. The possibility is also present, of course, in the case of the heart as in the case of the vascular reactions, that the concentration of epinephrin may be increased by stimulation of the central end of a peripheral nerve without any increase in the rate of the discharge per unit of time.

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[2] In our experience, in the cat (von Anrep worked with dogs) a not inconsiderable acceleration of the denervated heart can usually be obtained by stimulating the central end of the sciatic or the peripheral end of the splanchnic, even when the adrenal veins are clipped. There is nothing strange about this. It is obviously dependent upon the better blood flow through the coronary vessels. Guthrie and Pike (9) showed that in the perfused mammalian heart the rate could be increased decidedly by increasing the pressure of the perfusion fluid.

The question of the physiological value attributed by von Anrep to the reactions of epinephrin which he has so carefully studied, especially the question of the physiological value of the heart reactions is unaffected by substituting for the supposed reflex augmentation of the output of epinephrin an automatic redistribution of the blood which, without any material change in the rate of output, carries with it an increased supply of epinephrin to organs not involved in the vasoconstriction associated with the rise of blood pressure. We suggest that in such redistributions of blood containing the epinephrin secreted by the adrenals at a relatively stable and constant rate, rather than in a sudden outpouring of epinephrin, is to be sought the mechanism of any physiological effects of this type exerted by the naturally secreted epinephrin on the organism.

To return to the question of the relation of the adrenals to piqûre hyperglycemia, Kahn and Starkenstein (11) availed themselves in some experiments of the now well established fact that a certain proportion of rabbits deprived of both adrenals survive for a long time or indefinitely, in good health. In our experience the proportion is something like 20 per cent. If the animals which survive some weeks be included, it is greater. The statement of Freund and Marchand (12) that after complete extirpation of both adrenals rabbits die without exception in a short time, and usually on the first day, must be based on some error of operative technique. Unfortunately Kahn and Starkenstein contented themselves with tests, mostly qualitative, for sugar in the urine. They made no quantitative estimations of the blood sugar which in an investigation of this sort cannot be satisfactorily replaced by the qualitative tests on the aqueous humor employed by Kahn. Also in some of the very few relevant protocols published by them it is seen that practically no urine was secreted after the piqûre, and a negative sugar test in such cases would of course possess no value. It cannot therefore be admitted that the negative results of piqûre obtained by these writers on rabbits deprived of the adrenals have by any means settled the question of the indispensability of the epinephrin secretion for piqûre hyperglycemia.

The experiments of Biberfeld (13) are even less convincing and he admits that he does not now think that observations on sugar in the urine, the only observations he made, are satisfactory in the absence of blood sugar estimations.

The investigations of Jarisch (14) are free from this objection but are nevertheless open to criticism for other reasons. He endeavored to show that in rabbits after complete section of all the possible nervous paths from the central nervous system to the liver, piqûre was still followed by hyperglycemia when nervous connections of one adrenal were left whereas it did not cause hyperglycemia when the innervation of the adrenals was completely severed, that of the liver being intact. He estimated the blood sugar by Bertrand's method, precipitating the proteins by Schenck's method. Unfortunately he contented himself with a single blood sample, taken some time after piqûre. He compared the sugar content of this sample with a theoretical normal level and not, as we have invariably done, with that of a preliminary sample taken from the same animal. This renders the classification of some of the results as hyperglycemia quite arbitrary. In one series of ten experiments, for example, in which the innervation of the left adrenal was preserved while the remaining splanchnic distribution was severed, he counts a blood sugar content of 0.164 per cent among the hyperglycemias, very likely quite correctly. This is the series of experiments designed to show that augmented epinephrin secretion can cause hyperglycemia without the intervention of the hepatic nerves. In another series of five experiments in which the right adrenal was extirpated and all the nerves of the left adrenal cut, he classifies a blood sugar content of 0.167 per cent not as a hyperglycemia but as at "the upper limits of the normal content." This series was designed to show that in the absence of epinephrin secretion by the adrenals piqûre does not cause hyperglycemia. It must further be objected that in very few of Jarisch's experiments was the interval after the primary operation sufficiently long to permit a great accumulation of glycogen in the liver, even although the animals received cane sugar by stomach tube some time before the piqûre was made. The proof of this is the very low glycogen content of the liver in most of the experiments, even making allowance for the loss of glycogen in successful piqûre observations. For example, in three experiments in which he states that there was no hyperglycemia and in which therefore the glycogen content of the liver at the end of the experiment probably did not differ much from the content just before the piqûre, the glycogen only amounted to 0.7, 1.1 and 1.3 per cent, respectively. In five of the experiments with hyperglycemia in which the liver glycogen was estimated at the end, the percentages were 0, 0.2, 0.3, 0.8 and 2.1. With a good initial content of glycogen in our experiments the residual content after hyperglycemia produced by piqûre and later on by asphyxia was much greater, although the animals were not killed for a considerably longer time after the piqûre than appears to have been the case in the experiments of Jarisch.

The series of five experiments which are supposed to prove the inefficiency of the piqûre when the adrenals have been denervated although the liver nerves are intact is, we believe, completely vitiated on account of the low initial glycogen content. The percentages at the end of the experiments were 0, 0.5, 0.8, 0.9, 1.4. If there was no hyperglycemia, as Jarisch concludes, these percentages are probably not very different from the percentages before piqûre. It is vain to argue on the basis of one or two control experiments that with 1 per cent of glycogen in the liver piqûre will normally succeed. In our experience there is considerable variability in this matter in normal rabbits and there does not seem to be any reason why the production of a distinct piqûre hyperglycemia should depend solely upon the percentage of glycogen in the liver. At least one other factor, the rate of consumption of sugar in the animal, is obviously concerned. Even as regards the rate of mobilization of the glycogen it is improbable that the percentage of this substance in the liver should be the sole determining factor, and it cannot be assumed that every animal will respond in the same way to a given procedure when the glycogen store reaches a given level. The only way to be sure that a negative result is not due to too small a glycogen store is to work with animals whose livers are well filled with glycogen, and then always to make a glycogen estimation. It is impossible to decide beforehand that this or that animal will have enough glycogen to render a positive result certain after piqûre, particlarly when the animal has been recently subjected to a major operation. It may also be pointed out that positive results are much more important than negative ones in a question of this kind, and that only a decided hyperglycemia should be accepted as a positive result. Negative results in animals whose glycogen content is low ought not to be used at all.

A further criticism of these experiments is that it is surely difficult to be certain that the whole nerve supply of the liver has been divided by such an operation as that practiced by Jarisch. Finally, he made the piqûre under ether anesthesia. Even if the anesthetic was administered only during the operation, how is one to be certain that a subsequent hyperglycemia was not due to the anesthesia rather than to the piqûre? We have already shown (15) that in cats a brief administration of ether is capable of causing distinct hyperglycemia after the secretion of epinephrin has been abolished.

Among recent workers who have denied the importance of the adrenals in puncture hyperglycemia or glycosuria may be mentioned Wertheimer and Battez, Freund and Marchand and Trendelenburg and Fleischhauer. Wertheimer and Battez (16) found glycosuria in three cats following piqûre after removal of both adrenals. The experiments, however, were necessarily acute. In one case the animal was anesthetized with chloroform and, as the authors point out, it was impossible to discriminate between the effect of the anesthetic and the effect of the piqûre. In the other two cats spinal anesthesia (cocain) was employed. In five cats no definite glycosuria could be demonstrated. Blood sugar estimations were not made.

Freund and Marchand (17) conclude that the influence of piqûre is exerted directly on the liver and not through the adrenals. However, although they made numerous experiments on rabbits, none of them are entirely satisfactory. All were acute experiments, the piqûre being performed two or three hours after the removal of the adrenals, and it is difficult if not impossible in many cases to disentangle any effect of piqûre on the blood sugar from the effects of the operation and the anesthetic. The blood sugar was estimated by Bang's micro-method.

Trendelenburg and Fleischhauer (18) reached the conclusion that puncture glycosuria is not due to a "hormone" action of adrenalin discharged from the adrenals, since the rate of the discharge is not increased by the puncture. This result, however, is not arrived at by direct assay of the epinephrin coming from the adrenals, but is deduced from the fact that sugar puncture does not cause, in an anesthetized animal, a rise of blood pressure, whereas the minimal quantity of adrenalin which must be injected into a vein in order to elicit adrenalin glycosuria causes a distinct increase of blood pressure. Jarisch (14) has criticised, justly in our opinion, the deductions of Trendelenburg and Fleischhauer. For one thing, they relied entirely upon testing for sugar in the urine and did not estimate the blood sugar, the really important point. Their main argument is based upon premises by no means beyond question, and we believe that although their conclusion—that it is not an augmented epinephrin secretion which is responsible for puncture glycosuria—is correct, this cannot be established by such experiments.

Our own experiments were made on rabbits. The adrenals were removed at separate times. The interval between the first and second operation varied from eleven days to eight months. The piqûre was made ten days to eighty-one days after removal of the second adrenal. The floor of the fourth ventricle was exposed according to Eckhard's method, under local anesthesia by ethyl chlorid. A sample of blood was taken, usually from an ear-vein, before piqûre; a second sample about one to one and one-half hours after piqûre. About an hour later a third sample was drawn in order that some idea of the duration of the hyperglycemia and the maximum blood sugar content attained might be gotten. Finally asphyxia was produced by covering the mouth and nose at intervals for a period of fifteen to twenty minutes. The effect of the asphyxia on the heart-beat was sedulously controlled and a few free respirations allowed as soon as the heart was distinctly slowed, so that the asphyxia was never pushed to the point where life was in danger. A blood specimen was then drawn. The object of the asphyxia was to test whether, in the event of the piqûre yielding a negative result, a decided hyperglycemia was capable of being produced in any particular animal. We have already shown (15) that in cats with one adrenal removed and the secretion of epinephrin from the other abolished by section of its nerves, asphyxia invariably causes hyperglycemia when the glycogen content of the liver is not deficient. The animal was then killed and the glycogen content of the liver estimated by Pflüger's method, the sugar after hydrolysis of the glycogen being determined by Bertrand's method. The blood sugar was estimated by the method of Lewis and Benedict. Pearce's autoclave modification and the graduated test-tubes used by us in our previous work (1) were employed. It was recognized, of course, that when hyperglycemia had been produced by any of the procedures employed, the glycogen content as determined at the end of the experiment must be materially less than would have been found had the animal been killed at once. Accordingly before undertaking the piqûre experiments, we made a series of observations on the glycogen content of the liver under various diets, in cats whose epinephrin output had been interfered with by the operation mentioned and in rabbits and rats which had survived the removal of both adrenals. These experiments will be given in the second part of the paper.

The following condensed protocols illustrate the effects of piqûre and asphyxia on the blood sugar content in rabbits deprived of the adrenals.

Protocol. Rabbit 155, male

February 15, 1917. Left adrenal excised.

June 8, 1917. Weight, 2.625 kgm.

September 13, 1917. Right adrenal excised. From this time fed daily with carrots in addition to the ordinary diet (oats and hay daily, a carrot or a small quantity of green food once a week).

November 2, 1917. Weight. 3.275 kgm.

11.30 a.m. Obtained from ear normal blood specimen. It contained 0.102 per cent dextrose.

11.45 a.m. Piqûre.

12 30 p.m. Blood specimen from ear contained 0.205 per cent dextrose.

1.50 p.m. Blood specimen from ear contained 0.134 per cent dextrose. Asphyxia for 25 minutes, then at

2.20 p.m. Obtained blood from external saphenous vein, containing 0.216 per cent dextrose.

2.20 p.m Killed by heart-stab, removed liver. Glycogen in liver, 7.40 per cent.

Autopsy. Accessory adrenals not found. First piqûre a little above calamus and slightly to left of mid line; second piqûre about 10 mm. below the opening of the iter in mid line.

Protocol. Rabbit 181, male

November 19, 1917. Right adrenal excised.

November 30, 1917. Left adrenal excised. Weight 2.66 kgm. Some cane sugar was added to the drinking water from this date on. Otherwise, the ordinary diet.

February 19, 1918. Weight, 2.48 kgm.

12.30 p m. Normal blood specimen from ear contained 0.119 per cent dextrose.

12.50 p.m. Piqûre.

2.40 p.m. Blood from external jugular vein contained 0.349 per cent dextrose.

4.00 p.m. Blood from external jugular vein contained 0.449 per cent dextrose. Now caused asphyxia for 20 minutes and at

4.30 p.m. Blood obtained by cutting neck vessels, contained 0.517 per cent dextrose.

Liver at once excised; it contained 2.44 per cent glycogen. Taking the weight of the liver as 60 grams and the weight of the blood as 200 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar content to 0.52 per cent would correspond to 1.3 per cent of the liver weight. Therefore the initial content of glycogen must have been at least 4 per cent and no doubt considerably more.

Autopsy. Accessory adrenals not found. The piqûre was about 6 mm. above the calamus in the mid line.

Protocol. Rabbit 188, female

November 19, 1917. Excised right adrenal.

November 26, 1917. Gave birth to five young.

February 13, 1918. Excised left adrenal. Weight, 2.2 kgm.

Diet, cane sugar in drinking water and carrots given daily for four weeks prior to experiment, in addition to the ordinary diet.

March 12, 1918. Weight, 2.12 kgm.

10.40 a.m. Normal specimen from the ear contained 0.102 per cent dextrose.
11.00 a.m. Piqûre.
12.10 p.m. Blood from external jugular vein contained 0.161 per cent dextrose. Voided urine. Test with Fehling negative.
12.55 p.m. Blood from external jugular vein contained 0.176 per cent dextrose. Now caused asphyxia for twenty minutes.
1.15 p.m. Blood from jugular vein contained 0.262 per cent dextrose. Liver at once excised. It contained 2.35 per cent glycogen.

Autopsy. Piqûre 4 mm. above calamus in mid line. No accessory adrenals found in abdomen.

In these animals a decided hyperglycemia was caused by piqûre. The same is true of asphyxia following the piqûre. In the first experiment the piqûre hyperglycemia had distinctly diminished before asphyxia was induced, and after asphyxia the blood sugar content rose to fully the maximum level obtained in the first specimen after piqûre. In the second experiment, the second blood specimen taken after piqûre contained 0.1 per cent more dextrose than the first piqûre specimen. In spite of the high grade of the piqûre hyperglycemia, the blood sugar content increased still further during asphyxia. In the third experiment where the hyperglycemia after piqûre, although quite distinct, was not so great as in the second, the specimen drawn after asphyxia also showed a decided increase in the blood sugar as compared with the second specimen after piqûre. There can be no question, then, that piqûre, like asphyxia, is capable of causing hyperglycemia in rabbits after removal of the adrenals. Obviously, as already pointed out, in a question of this kind positive results are much more important than negative ones. In the three animals the liver was well filled with glycogen, a considerable period having elapsed since the last operation.

Negative results in animals taken at too short an interval after the adrenal operation and containing little glycogen in the liver, have of course no weight at all. Not a few of the observers who have denied the possibility of producing hyperglycemia by piqûre after adrenalectomy have been misled by want of attention to this point. The following protocols are samples of our negative experiments on adrenalectomized rabbits:

Protocol. Rabbit 156, male

March 20, 1917. Left adrenal excised.

June 8, 1917. Weight, 1,775 kgm.

September 15, 1917. Right adrenal excised. From this time on the animal was fed regularly with carrots in addition to the ordinary diet.

November 13, 1917. Weight, 2.45 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.101 per cent dextrose.
11.35 a.m. Piqûre. Two stabs.
12.15 p.m. Blood from cut external saphenous vein contained 0.107 per cent dextrose.
1.10 p.m. Blood from femoral vein contained 0.097 per cent dextrose. Now caused asphyxia for twenty minutes and at
1.40 p.m. Obtained blood specimen from external jugular vein, containing 0.121 per cent dextrose.

Immediately killed and excised liver, which contained 1.02 per cent of glycogen.

Autopsy.—Accessory adrenals not found. The first piqûre was 3 to 4 mm. above the calamus in the mid line, the second 6 to 7 mm. above the first in the mid line.

Protocol. Rabbit, 183, male. Weight, 2.235 kgm.

December 7, 1917. Right adrenal excised. Weight, 2.24 kgm.

Feb. 11, 1918. Left adrenal excised. Weight, 2.20 kgm. Kept on ordinary diet from this date, except that some cane sugar was given in the drinking water one day before the piqûre experiment. The weather was very cold at this time.

February 21, 1918. Weight, 2.235 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.114 per cent dextrose.
11.10 a.m. Piqûre.
12.10 p.m. Blood specimen from ear contained 0.131 per cent dextrose.
1.20 p.m. Blood from ear contained 0.121 per cent dextrose. Asphyxia was now caused for twenty-five minutes, then at
1.50 p.m. Obtained blood specimen from external jugular, containing 0.128 per cent dextrose.

The neck vessels were now severed and a blood specimen obtained which contained 0.126 per cent dextrose. The liver was at once excised; its glycogen content was 2.32 per cent.

Autopsy. A small accessory adrenal was found under the left renal vein. The piqûre was 6 to 7 mm. above the calamus in the mid line.

It would not be profitable to speculate on the reason for the absence of piqûre hyperglycemia in these animals. Since precisely similar negative results may be obtained in normal animals, we do not see how they can be connected with the presence or absence of the adrenals. While everybody is agreed that a high glycogen content is favorable for the occurrence of piqûre hyperglycemia, and that a very low glycogen content is incompatible with it, there is no evidence as already stated, that hyperglycemia must necessarily be obtained in an animal with more than a certain percentage of glycogen in its liver, whereas in animals with less than that percentage it can never be obtained.

The following protocols illustrate the results on normal rabbits with the same technique as that employed for the adrenalectomized animals:

Protocol. Rabbit 186

Male, which had been long in stock. Weight, 1.80 kgm. Ordinary diet.

February 27, 1918.

11.00 a.m. Normal blood specimen from ear contained 0.107 per cent dextrose.
11.15 a.m. Piqûre.
12.15 p.m. Blood from ear contained 0.374 per cent dextrose.
1.20 p.m. Blood from ear contained 0.46 per cent dextrose. Asphyxia was now caused for twenty-five minutes and at
1.45 p.m. A blood specimen was obtained with 0.514 per cent dextrose.

Ten minutes later the blood vessels in the neck were severed and another blood specimen obtained with 0.52 per cent dextrose. The liver was immediately excised. Its glycogen content was 2.96 per cent. The liver weighed 61 grams. Taking the total blood as 140 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar to 0.52 per cent would represent almost 1 per cent of the liver weight. The glycogen content before piqûre must therefore have been at least 4 per cent and no doubt was considerably more.

Autopsy. The piqûre was 5 mm. above the calamus in the mid line.

Protocol. Rabbit 182

Normal female from the stock. (Control for rabbit 181, and on the same diet).

February 19, 1918. Weight, 2.63 kgm.

12.10 p.m. Normal blood specmen from ear contained 0.11 per cent dextrose.
12.30 p.m. Piqûre.
1.40 p.m. Blood from ear contained 0.367 per cent dextrose.
3.00 p.m. Blood from ear contained 0.308 per cent dextrose. Asphyxia was now caused for twenty minutes and at
3.20 p.m. A blood specimen obtained from the ear contained 0.367 per cent dextrose.

The animal was now bled from the throat and a specimen obtained at 3.30 p.m. with 0.417 per cent dextrose. The glycogen content of the liver was 2.04 per cent. If only the amount of glycogen which must have been mobilized to make up a blood sugar content of 0.42 per cent be added, the liver must have contained before the piqûre experiment at least 3 per cent of glycogen and doubtless it contained more.

Autopsy. The piqûre was 4 to 5 mm. above the calamus in the mid line.

The following two protocols illustrate the negative results of piqûre obtained on normal rabbits just as on adrenalectomized rabbits.

Protocol. Rabbit 187, male

March 11, 1917. Weight, 1.41 kgm. No food given (except water) for two days prior to experiment.

10.00 a.m. Normal specimen of blood from ear contained 0.124 per cent dextrose.

10.15 a.m. Piqûre.

11.15 a.m. Blood from external jugular vein contained 0.136 per cent dextrose.

12.15 p.m. Blood from external jugular vein contained 0.130 per cent dextrose. Now caused asphyxia for twenty minutes.

12.35 p.m. Blood from external jugular vein contained 0.142 per cent dextrose.

Severed blood vessels in neck and bled to death. A specimen of this mixed blood contained 0.151 per cent dextrose. The liver was at once excised; it contained 0.34 per cent glycogen.

Autopsy. The piqûre was 6 mm. above the calamus in the mid line.

Protocol. Rabbit 157

Normal female from stock. It had carrots regularly in addition to the ordinary diet for two weeks before the piqûre experiment.

November 13, 1917. Weight, 2.0 kgm.

11.15 a.m. Normal blood specimen from ear contained 0.122 per cent dextrose.

11.50 a.m. Exposed and opened the occipito-atlantoid membrane under local ethyl chlorid anesthesia, as in all the other experiments; but did not perform piqûre as yet.

12.40 p.m. Blood specimen fom ear contained 0.116 per cent dextrose.

12.55 p.m. Piqûre made (two stabs).

2.10 p.m. Blood from femoral vein contained 0.10 per cent dextrose. Asphyxia was now induced for twenty minutes and at

2.30 p.m. A blood specimen was obtained from the external jugular vein with 0.101 per cent dextrose.

The animal was at once killed by heart stab. The liver contained only a trace (less than 0.05 per cent) of glycogen.

Autopsy. First piqûre, 3 to 5 mm. above the calamus in the mid line, second piqûre, 7 to 8 mm. above the first and a little to the left of the mid line.

The object of the experiment just cited was to control the effect of the operation as such, apart from the piqûre, on the blood sugar. The experiment failed for this purpose because of the low glycogen content of the liver (in spite of the carrot diet) which did not permit either the subsequent piqûre or asphyxia to cause any hyperglycemia. The following protocol gives the data of an experiment of the same kind, but on a rabbit whose liver was well filled with glycogen. It will be seen that the operation as such, with any attendant emotional excitement, caused no hyperglycemia.

Protocol. Rabbit 189, male

Diet. Sugar in drinking water and carrots for five days prior to the experiment, in addition to the ordinary diet. The animal took the sugared water readily. Weight 2.0 kgm.

9.55 a.m. Normal blood specimen from ear vein contained 0.126 per cent dextrose.

10.15 a.m. Under local anesthesia (ethyl chlorid) exposed floor of fourth ventricle but did not perform piqûre as yet. Sutured the wound.

11.20 a.m. Blood specimen from ear vein contained 0.124 per cent dextrose.

11.30 a.m. Piqûre performed.

12.30 p.m. Blood specimen from ear vein contained 0.249 per cent dextrose. Asphyxia was now caused for twenty minutes and at

1.00 p.m. A blood specimen was obtained from the external jugular vein. It contained 0.343 per cent dextrose. Urine voided at this time gave a marked reduction with Fehling's solution. The animal was killed by bleeding from the neck vessels. The liver, excised immediately, weighed 82 grams and contained 7.14 per cent of glycogen.

Autopsy. The piqûre was 4 mm. above the calamus in the mid line.

The results of a number of preliminary experiments on normal rabbits in which glycogen determinations were not made, are given in table 1, in order to emphasize the point that a negative piqûre experiment in an adrenalectomized animal must not be attributed off hand to the absence of the adrenals. The six rabbits had lived a long time in the laboratory under identical conditions and on the same (ordinary) diet. Carrots were added one day before the experiment. It will be seen that three of the animals yielded distinctly positive results with piqûre. In two the result was negative, in one doubtful.

TABLE 1

Percentage of blood-sugar

NORMAL	PIQÛRE	ASPHYXIA
0.135	0.162	0.163
0.125	0.390	
0.130	0.134	
0.110	0.263	
0.101	0.178	
0.102	0.115	0.122

TABLE 2

Adrenalectomized rabbits

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	ADRENALS EXCISED		PERCENTAGE OF GLYCOGEN IN LIVER	REMARKS
		First	Second		
1917 October 23	145	*1917* January 18	*1917* September 17	6.42	Carrots daily since last operation in addition to usual diet*
November 2	155	February 15	September 13	7.40	Same as for 145
November 13	156	March 20	September 15	1.00	Same as for 145
November 5	158	*1916* December 7	September 22	0.68	Attempted piqûre. Died; liver taken after one-half hour
1918 February 19	181	*1917* November 19	November 30	2.44	Cane sugar daily since last operation, in addition to ordinary diet
February 21	183	December 17	*1918* February 11	2.32	Cane sugar one day before experiment (ordinary diet). Very cold weather
February 25	184	December 7	February 13	Trace	Ordinary diet. Rabbit has mange, not eating well, losing weight
March 12	188	November 19	February 13	2.35	Cane sugar and carrots daily since last operation, in addition to ordinary diet

* Rabbit 145 was sacrificed for glycogen estimation. In all the others a piqûre experiment was done before the liver was excised. The ordinary diet for rabbits consisted of oats and hay daily, with a carrot or a small piece of green food once a week.

PART II. RELATION OF THE ADRENALS TO THE GLYCOGEN CONTENT OF THE LIVER

Several of the writers on the problem of the possibility of producing experimental glycosurias and hyperglycemias after removal of the adrenals have raised the question whether the negative result might not be due to inability of the liver to form or to store glycogen in the adrenalectomized animal. Since as shown above the result is not negative, this question does not arise. Nevertheless, before making our observations on piqûre and in the course of them, we made a considerable number of experiments on cats, rabbits and rats, in order to obtain information upon the glycogen content of the liver in animals in which the adrenals had been removed or, in the case of the cats, the secretion of epinephrin interfered with. The rabbits used had survived the removal of the second adrenal eleven days to nine months, when killed for the glycogen determination. Details as to diet, glycogen content, etc., are given in table 2, which includes a certain number of animals in which the glycogen was determined after hyperglycemia had been produced by piqûre and by asphyxia (see protocols in part I.). The glycogen, estimated at the end of these experiments must, of course, be less than the actual content before the piqûre.

TABLE 3

Normal control rabbits

DATE	NUMBER OF ANIMAL	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917			
October 23	144	2.80	Ordinary diet
October 29	150	4.73	Ordinary diet with addition of carrots for 6 days prior to experiment
November 22	160	2.58	Ordinary diet with addition of carrots for one week prior to experiment
November 13	157	Trace*	Ordinary diet with addition of carrots for one week prior to experiment. (Piqûre, etc.)
1918			
February 19	182	2.04	Cane sugar daily in addition to ordinary diet since November 30, 1917 (Piqûre, etc.)
February 27	185	1.74	Ordinary diet. Piqûre attempted. Died
February 27	186	2.96	Ordinary diet. (Piqûre, etc.)
March 11	187	0.34	Starved two days. Piqûre (negative)

* Less than 0.05 per cent.

In table 3 are displayed the results of a number of glycogen determinations on normal control rabbits. It will be seen that there is no material difference between the results in table 2 and table 3.

Since cats do not survive the removal of both adrenals, we excised one adrenal and divided the nerves of the other so as to abolish or reduce greatly the output of epinephrin. Protocols of two such experiments follow:

Protocol. Cat 125, male

Diet. Liver, milk, occasionally fish and a daily ration of rice or potatoes boiled with milk.

August 18, 1917. Weight, 2.64 kgm. Excised right adrenal. It weighed 0.266 gram and contained 0.12 mgm. epinephrin. Extirpated left semilunar ganglion. Excised left superior cervical ganglion.

September 18, 1917. Weight, 1.92 kgm. Left pupil contracted and nictitating forward.

11.25 a.m. Urethane 3 grams (stomach).

2.50 to 3.15 p.m. Inserted tracheal and jugular cannulae; artificial respiration; coeliac and mesenteric arteries tied and a lobe of liver at once excised for glycogen estimation. Completed cava pocket in usual manner.

3.15 p.m. Both pupils equal and nictitating membranes slightly forward.

3.20 p.m. Pocket experiment two minutes. No eye reactions.

3.24 p.m. Pocket experiment five minutes. No eye reactions.

3.30 p.m. Pocket experiment nine minutes. No eye reactions.

3.44 p.m. 0.5 cc. 1: 1,300,000 adrenalin. Very good pupil dilatation in 11.2 seconds. No nictitating reaction.

3.48 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Good pupil reaction in 11.4 seconds. No nictitating reaction.

3.53 p.m. 0.5 cc. 1: 4,000,000 adrenalin injected: Small pupil reaction in 13.8 seconds. No nictitating reaction.

3.58 p.m. 0.5 cc. 1: 5,200,000 adrenalin injected. Small pupil reaction in 13.6 seconds. No nictitating reaction.

Now collected the following blood samples from the adrenal (short pocket):

Sample 1, 1.4 grams in one minute (1.4 grams per minute).

Sample 2, 4.4 grams in four minutes (1.1 grams per minute).

Sample 3, 3.8 grams in eight minutes (0.475 gram per minute).

Obtained blood from abdominal aorta. Left adrenal weighed 0.257 gram, and contained 0.17 mgm. epinephrin. Glycogen in lobe of liver removed at beginning of experiment, 4.26 per cent.

The epinephrin assay of the adrenal vein blood gave the following results:

The tests with rabbit intestine and uterus segments showed that even the third adrenal specimen of blood could not have contained more than 1: 260,000,000 epinephrin, corresponding to an output of

0.0000018 mgm. per minute for the animal or 0.0000007 mgm. per minute per kilogram of body weight; i.e., not more than one-three hundred and fiftieth of the normal output as estimated by the segments.

The second adrenal specimen did not give a much greater effect than the indifferent arterial blood and a far smaller effect than indifferent blood containing 1: 260,000,000 adrenalin.

The eye reactions were negative even when blood was collected in the cava pocket for nine minutes. Yet distinct reactions were obtained when 0.5 cc. of a 1: 5,300,000 solution of adrenalin was injected. Accordingly, as tested in this way, the output could not have amounted to 0.00001 mgm. per minute for the animal or 0.000004 mgm. per kilogram of body weight per minute; i.e., not one-one hundred and fiftieth of the normal, as estimated by eye reactions.

Protocol. Cat 113, male

Same diet as for cat 125.

July 20, 1917. Weight, 2.725 kgm. Excised right adrenal. It weighed 0.152 gram and contained 0.16 mgm. epinephrin. Extirpated left semilunar ganglion and severed lumbar chain below the diaphragm.

August 16, 1917. Weight, 2.545 kgm. Right splanchnics divided.

August 30, 1917. Weight, 2.71 kgm. Blood sugar tests made as follows: Normal specimen, 0.089 per cent; specimen collected after frightening, 0.084 per cent; specimen collected after asphyxia, 0.153 per cent.[3]

August 31, 1917. Excised left superior cervical ganglion.

September 18, 1917. Weight, 2.41 kgm. Condition excellent.

10.15 a.m. 5 grams urethane (stomach).

11.50 a.m. Inserted tracheal and jugular cannulae; tied coeliac and mesenteric arteries; at once clamped off and removed the left and part of the middle lobe of the liver for glycogen estimation. Then completed a cava pocket in the usual way.

12.20 p.m. Left pupil wider than right; both nictitating slightly forward.

12.20 p.m. Pocket experiment two minutes. Small pupil and nictitating reactions in 10 seconds.

12.24 p.m. Pocket experiment, one minute. Small pupil and nictitating reactions in 13.2 seconds.

12.26 p.m. Pocket experiment, three minutes. Small pupil and slight nictitating reactions in 11 seconds. (Not much different from observation at 12.20.)

12.34 p.m. 0.5 cc. 1: 1,300,000 adrenalin injected. Very large pupil and nictitating reactions in 6.2 seconds.

12.40 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Very large pupil and nictitating reactions.

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[3] The blood sugar results of this experiment have been already cited in our paper in this Journal, 1917, xliv, 543.

12.41 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Same result.
12.46 p.m. 0.5 cc. 1: 2,000,000 adrenalin injected. Very good pupil reaction in 6 seconds. (Nictitating still back.)
12.48 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Good pupil reaction in 8.2 seconds.
12.50 p.m. 0.5 cc. 1: 4,000,000 adrenalin injected. Probably slightly larger reaction than that produced by blood collected for two to three minutes in the cava pocket.

Now collected blood specimens from adrenal (short pocket) as follows:
Sample 1, 1.2 grams in 35 seconds (2 grams per minute).
Sample 2, 7.0 grams in four and one-half minutes (1.55 grams per minute).
Sample 3, 9.0 grams in nine minutes (1 gram per minute).

Collected blood from jugular vein and also from abdominal aorta. Left adrenal weighed 0.141 gram and contained 0.15 mgm. epinephrin. Glycogen in liver removed at beginning of experiment, 4.75 per cent.

The eye reactions in this animal indicated that the output of epinephrin could not have been more than 0.00004 mgm. per minute for the animal or 0.000017 mgm. per kilogram of body weight per minute; i.e., not more than one-thirty-fifth of the normal output, as estimated by eye reactions. The rabbit uterus and intestine segment tests showed that the third adrenal specimen contained about 1:65,000,000 epinephrin, corresponding to an output of 0.000015 mgm. per minute for the animal or 0.000006 mgm. per kilogram of body weight per minute; i.e., one-fortieth of the normal as estimated by the segments.

The glycogen content of the liver in these two cats was 4.26 and 4.75 per cent, respectively. In two normal control cats, in which the operative procedure followed in cats 125 and 113 for obtaining a lobe of the liver was imitated under urethane, with ligation of the coeliac and mesenteric arteries, the glycogen content of the liver was 1.95 and 2.55 per cent respectively. In a third normal cat, killed instantaneously without urethane, the content was 4.13 per cent.

Since, as is known, a large proportion of rats survive the excision of both adrenals we made some observations on these animals also. It has been stated by Schwarz (19) that rats do not survive more than a day if both adrenals are removed at one time. He therefore left an interval between the two operations. He is certainly mistaken in this matter for we excised the two adrenals at one time in thirteen rats. Of these eight died in one to fourteen days. The remaining five recovered completely and were sacrificed for the glycogen estimation. The results are given in table 4. In table 5 are given for comparison the glycogen percentages found in five normal rats and in three rats on which a laparotomy had been performed in order to control approxi-

TABLE 4

Adrenalectomized rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	DATE OF ADRENALECTOMY	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917		*1917*		
November 13	159	October 28	5.01	Ordinary diet*
November 22	162	October 28	2.41	Ordinary diet
December 14	165	December 3	Trace	Unleavened bread and milk; lost weight; accessory adrenal found
December 24	167	December 11	1.40	Ordinary diet (no milk for two days before experiment). Small accessory adrenal found
1918				
January 4	169	December 11	2.99	Unleavened bread, water, no milk; butter for four days

* The ordinary diet for rats consisted of bread, corn, oats and milk daily and a small piece of cabbage once a week.

TABLE 5

Normal Rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917			
November 22	161	2.69	Carrots and sugar for 1 week before experiment in addition to ordinary diet
November 22	163	1.48	Ordinary diet
December 14	166	2.59	Unleavened bread and milk
December 29	168*	2.32	Ordinary diet but no milk for two days before experiment
1918			
January 4	170*	3.98	Unleavened bread, water but no milk (butter four days prior to experiment)
January 25	171*	4.45	Only unleavened bread and water for two weeks before experiment
January 25	172	5.19	Only unleavened bread and water for two weeks before experiment

* To control any general effects of the operation in the adrenalectomized rats, a laparotomy was performed on these three normal rats on December 19, 1917. Adrenalectomy was not performed but otherwise the operation was similar.

mately the effects, as regards trauma, anesthesia, etc., of the operation on the adrenalectomized rats but without removal of the adrenals. No essential difference is shown in the two tables.

Schwarz has stated that the livers of adrenalectomized rats after feeding with dextrose or cane sugar contained considerable quantities of glycogen. His protocols show no definite deficiency as compared with normal rats. On the other hand, he asserts that when carbohydrate is supplied in the form of starch as in feeding "Semmeln," the livers of the adrenalectomized rats are practically free from glycogen, while normal rats with the same diet show a good content. Although the fact that with sugar feeding the adrenalectomized animals form and store considerable quantities of glycogen is sufficient to exclude the idea that any essential change in the process of glycogenesis is caused by the removal of the adrenals, we made some experiments in order to control Schwarz's observations. Two adrenalectomized rats were fed from the time of the operation with a diet certainly free from added sugar, the "matzo" (matzoh), or unleavened bread used during the Jewish Passover. Of these rats, one had a glycogen content of 2.99 per cent. The control rat (170 in table 5) had a content of 3.98 per cent. The other adrenalectomized rat had only a trace of glycogen in the liver but the animal was losing in weight, had been apathetic for some days and was sacrificed because it was feared it was going to die. The control normal rat (166, table 5) had 2.59 per cent. Two other control rats (171 and 172, table 5), were fed solely on unleavened bread and water for two weeks. The glycogen contents were 4.45 per cent and 5.19 per cent respectively.

Kahn and Starkenstein (11) made a few experiments to test the results of Schwarz. They likewise state that rats do not survive when both adrenals are removed at one sitting. Indeed, according to them, if a shorter interval than three to four weeks is left between the first and second operations, death ensues within two days after the second operation. As we have already pointed out, this conclusion is certainly erroneous. A quite considerable proportion of rats survive for a much longer time after simultaneous removal of both adrenals. Kahn and Starkenstein state that adrenalectomized rats fed on a diet of milk, "Semmeln" and some oats, do not store glycogen in the liver except in traces. The number of experiments performed by them was very small and the few protocols given in their paper to not support this conclusion. In the three adrenalectomized animals in which glycogen determinations were made, the glycogen content in one was

2.38 per cent. In another, piqûre had been done before the liver was obtained for the glycogen determination and if a hyperglycemia had been produced the glycogen content before piqûre would doubtless have been considerably higher than that actually found. They did not make any estimations of blood sugar. The interval between the last adrenal operation and the glycogen determination was in general too short for the post-operative depletion of the glycogen store to be certainly made good, especially in view of the fact that they did not purposely feed sugar to the animals. We have had positive results even on a diet containing practically no sugar. That adrenalectomized rats will sometimes show only a trace of glycogen in the liver is true enough, but this is also the case with normal rats. It is necessary before comparing so-called control animals with the operated animals to know that the consequences of the operation as such on the glycogen store have been entirely eliminated. And this can never be assumed with absolute certainty in any particular animal, especially when only a few days have elapsed since the operation. Schwarz anesthetized the rats in order to administer sugar, etc., by the stomach tube and as he fed them in this way on three successive days the effects of the anesthesia may not have been entirely negligible.

SUMMARY

1. In rabbits which have survived the removal of both adrenals and have recovered from the operation, and whose livers are well filled with glycogen, piqûre causes decided hyperglycemia just as in normal rabbits. The hypothesis that piqûre hyperglycemia is caused in the same way as the hyperglycemia produced by injecting adrenalin, by an increased liberation of epinephrin from the adrenals into the blood, must therefore be abandoned. We have previously shown (in cats) that the hyperglycemia associated with asphyxia and with ether anesthesia is likewise not dependent upon the secretion of epinephrin. In the present research we have had many opportunities to observe that hyperglycemia is caused by asphyxia in adrenalectomized rabbits.

2. The results of previous observers who have failed to obtain piqûre hyperglycemia in rabbits after extirpation of both adrenals are due to the fact that they have performed the piqûre immediately after the adrenalectomy, or if an interval has been allowed it has been too short to permit complete recovery from the adrenal operation and the liver has been insufficiently stored with glycogen. Even when a consider-

able interval has elapsed after the adrenal operation, the state of nutrition of the animal or the diet has sometimes been unfavorable for glycogen accumulation and therefore a positive result could not be expected.

3. There is no real evidence that piqûre increases the rate of liberation of epinephrin from the adrenals.

4. It is pointed out that the reactions of denervated vascular regions and of the heart, isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, which have been interpreted as showing that the rate of liberation of epinephrin is increased by stimulation of afferent nerves and by asphyxia, have a different significance.

5. The formation and storing of glycogen in the liver is not affected by removal of both adrenals in rabbits, or by removal of one adrenal and section of the nerves of the other in cats with consequent abolition or marked reduction in the rate of liberation of epinephrin. In rats, also, extirpation of the adrenals produces no essential change in the capacity of the liver to form and store glycogen.

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NORMAL MECHANISM FOR THE CONTROL OF OXIDATION IN THE BODY

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Lavoisier, shortly after his discovery that oxygen supported combustion, showed that the ingestion of food increased oxidation in the body. Rubner found that of the foodstuffs, meat ingestion increased oxidation most, fat next and sugar least. Several theories have been advanced in attempts to explain how food increases oxidation in the body. Von Mering and Zuntz (1) believed that the increased oxidation following the ingestion of food was due to the increased activity of the intestinal tract. Voit (2), Rubner, Johansson (3) and Benedict (4) have shown that this explanation is not the correct one. Voit believed that the presence of increased quantities of food materials augmented the inherent power of the cells to metabolize. Rubner, on the contrary, contended that the fundamental metabolism of the cell was not effected by the ingestion of food but that the increased heat production which follows the taking of food was due to heat developed from intermediary reactions and oxidations which were in no way involved in the life processes of the cells. Benedict (5) claims that the ingestion of carbohydrates increases oxidation by the formation of acid intermediary products which stimulate metabolism. Lusk (6) holds that the stimulating effect of protein to increased heat production is due to the influx of amino acids which cause an increase in metabolism by their mass action on the protoplasm of the cells.

We (7) have shown that when catalase is increased by the stimulation of the different glands, particularly the liver, to an increased output of this enzyme, there results an increase in oxidation in the body and that when there is a decrease in catalase, a decrease in oxidation follows. The object of the present investigation was to determine if the ingestion of food increases the catalase of the blood and hence of the tissues parallel with the increase in heat production, and if so, how this is brought about and whether protein is more effective in this respect than fat or carbohydrate. Dogs, rabbits and cats were used in the investigation. The form in which the foodstuffs were introduced and the method of introduction will be described later in the paper along with the description of the experiments. Previous to the introduction of the food material at least two determinations were made o the catalase of the blood for the normal. After the introduction of the food materials determinations of the catalase were also made at fixed intervals. The determinations were made by adding 0.5 cc. of blood to a known amount of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. On account of the low catalase content of the blood of the dogs, 50 cc. of hydrogen peroxide were used while 250 cc. were used for the cats and rabbits. After the volume of gas collected as described in ten minutes had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken in a shaking machine at a fixed rate of one hundred and eighty double shakes per minute during the determinations.

The first part of this paper is concerned with showing that the introduction of the foodstuffs increases the catalase of the blood and hence of the tissues, and that this increase is brought about by the stimulation of the glands, particularly the liver, to an increased output of this enzyme. The fat used was 200 grams of olive oil emulsified by shaking with 100 cc. of a 1 per cent sodium carbonate solution; the sugar, 400 grams of dextose dissolved in 500 cc. of water, and the meat was in the form of a peptic digest. The digest was made by adding 800 grams of ground lean beef, previously freed as much as possible from fat and connective tissue, to 500 cc. of 0.5 per cent hydrochloric acid in which had been dissolved 100 grams of a commercial preparation of pepsin. The mixture was permitted to stand in a thermostat at 40°C. for twenty-four hours. After etherizing a dog that had gone without food for twenty hours, the abdominal wall was opened and the digest of 800 grams of lean meat was introduced into the stomach and intestines in about equal quantities. The acid of the digest was almost neutralized, previous to introduction into the animal, by the addition of sodium carbonate. The material was introduced under pressure through a piece of rubber tubing and a hypodermic needle which was inserted through the wall of the stomach and intestine respectively. In a similar manner the emulsified olive oil and sugar solution were introduced into other dogs. Determinations were made of the catalase of 0.5 cc. of the samples of blood taken from the portal vein, liver and external jugular vein before as well as at fixed intervals after the introduction of the materials. The blood was taken from the portal and jugular veins by means of a hypodermic needle attached to a 1 cc. pipette. The blood of the liver was collected from a superficial incision made in this organ. Blood thus collected was examined under the microscope and found to be free from liver cells. Bile taken from the gall bladder and tested for catalase was found to contain none. Hence any variation in the catalase content of the blood of the liver from that found elsewhere in the body could not be due either to liver tissue or bile.

In figure 1 curves *A*, *B* and *C* were constructed from data obtained from a dog previous to and after the introduction of the 200 grams of olive oil. Curve *A* was constructed from data obtained from determinations of the catalase in the blood of the liver; curve *B* in the blood from the portal vein and curve *C* in the blood from the external jugular. The figures (0 to 120) along the abscissa indicate time in minutes while the figures along the ordinate (0 to 130) indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood. It may be seen that 0.5 cc. of the samples of blood taken from the liver previous to the introduction of the olive oil liberated 80 and 78 cc. of oxygen respectively from hydrogen peroxide; that the blood from the portal vein liberated 75 and 75 cc. and that from the jugular 71 and 72 cc. Fifteen minutes after the introduction of the olive oil the blood from the liver liberated 101 cc. of oxygen, that from the portal vein 80 cc. and that from the jugular 75 cc.; fifteen minutes later, the blood from the liver liberated 105 cc. of oxygen, the portal blood 91 cc. and the blood from the jugular 76 cc.; after forty-five minutes the blood from the liver liberated 111 cc., that of the portal vein liberated 92 cc. and that of the jugular 82 cc.; and after sixty minutes the blood from the liver liberated 116 cc., that of the portal vein 94 cc. and that of the jugular 86 cc.; and after seventy-five minutes the blood of the liver liberated 125 cc.

From these data it may be seen that previous to the introduction of the olive oil the blood from the liver was richer in catalase than that of the portal vein and that the blood from the portal vein, in turn, contained more catalase than the blood from the jugular. It may also be seen that after the introduction of the olive oil the catalase of the blood from the liver increased more rapidly than did that of the portal vein, and the catalase of the blood of the portal vein more rapidly than

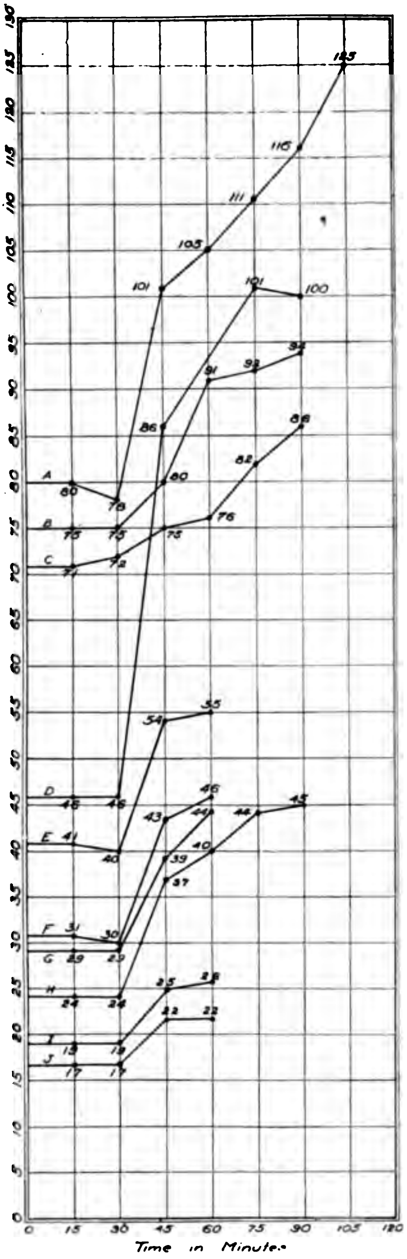

Fig. 1. Curves showing the effect of the introduction of olive oil, meat digest, peptone solution and dextrose into the alimentary tract on the catalase content of the blood; *A*, *B*, *C* the effect of the introduction of olive oil, *D* of meat digest, *E*, *F* and *G* of peptone, *H*, *I* and *J* of dextrose. The figures (0–120) along the abscissa indicate time in minutes; the figures (0–130) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

that of the jugular. The fact as shown in curve *B* that immediately after the introduction of the olive oil the catalase of the blood of the portal vein increased more rapidly than that of the jugular, is taken to mean that the gastric and intestinal glands as well as the liver were stimulated to an increased output of catalase by the olive oil. The preceding experiment was repeated on several other dogs and in general the same results were obtained except when smaller amounts of olive oil were used or for some unknown reason, the liver, gastric and intestinal glands were not so strongly stimulated, there was scarcely any change produced in the catalase of the blood of a systemic vein, such as the jugular, although the catalase of the blood of the liver and of the portal vein was always increased. We have found that the catalase of the blood of the systemic veins in general contain between 5 and 20 per cent less catalase than the blood taken directly from the liver or from one of the hepatic veins. This observation is interpreted to mean that while catalase is being given off by the liver, the gastric and intestinal glands, it is being used or destroyed by the tissues. In addition to stimulating the liver and the gastric and intestinal glands, it is probable that other glands, such as the pancreas and spleen, were also stimulated to an increased output of catalase by the olive oil just as we had found to be the case with alcohol.

The same method of procedure was used in studying the effect of the gastric digest, the dextrose and also a peptone solution, as was used in studying the effect of the emulsified olive oil. The peptone solution was made by dissolving 100 grams of peptone in 250 cc. of water. This solution was made acid to the extent of 0.3 per cent by the addition of hydrochloric acid. The results for the gastric digest are given in curve *D*, for the peptone in curves *E*, *F* and *G*, and for the dextrose in curves *H*, *I* and *J*. It may be seen that the effect of the gastric digest, the dextrose and the peptone was in general the same as that of the olive oil, namely, a production of an increase in catalase.

The second part of this paper is concerned with determining whether meat in keeping with its greater stimulating effect on heat production is more effective than fat or sugar in stimulating the glands, particularly the liver, to an increased output of catalase. The animal used was a dog. The foodstuffs were 100 grams of olive oil, emulsified by shaking with 50 cc. of a 1 per cent sodium carbonate solution; the sugar, 225 grams of dextrose dissolved in 400 cc. of water, and the meat, 800 grams of lean round steak freed as far as possible from connective tissue and fat. The emulsified olive oil and sugar solution were introduced by means of a stomach tube and the animal ate the 800 grams of steak which was finely ground. The catalase of 0.5 cc. of samples of blood taken from the external jugular before as well as at fixed intervals after the giving of the foodstuffs was determined according to the method described in the first part of the paper. There was an interval of about two weeks between the administration of each of the three foodstuffs and the same dog was used for all three of them. The effect of the different foodstuffs given in isodynamic quantities on the catalase content of the blood of the dog is shown in figure 2. The figures along the abscissa indicate time in minutes while those along the ordinate indicate percentage increase in catalase. The curve marked *meat* was constructed from data obtained from the dog after feeding the 800 grams of lean steak; the one marked *fat* after giving the 100 grams of olive oil and the one marked *sugar* after giving the 225 grams of dextrose. It will be seen that two hours after eating the meat the catalase of the blood had increased by 60 per cent; after three hours it had increased 81 per cent and after four hours 115 per cent at which time it had reached a maximum and then began to decrease and had returned almost to the normal amount after nine hours. It may be seen also that the increase produced in catalase by the fat reached a maximum of 60 per cent in three hours and had returned almost to normal after four and one-half hours, and that the effect of the sugar reached a maximum of 51 per cent in two and one-half hours. Comparing the results for the three foodstuffs it will be seen that meat ingestion produced the greatest increase in catalase, fat next and sugar least. This experiment was repeated on the same dog three different times and in general the same results were obtained. The foodstuffs were also fed in isodynamic quantities to several other dogs but none of these dogs gave as good result as those described above. No trouble was experienced in inducing these dogs to eat meat but they would vomit almost invariably after the introduction of either the olive oil or the sugar. In some instances we found, when using a dog whose blood catalase was high, that the feeding even of meat produced very little or no change in the catalase of the blood of the jugular. Upon etherizing and opening the abdominal wall of such a dog, however, and testing the blood taken either directly from the liver or from one of the hepatic veins, we always found the catalase of the blood of the liver to be higher by 50 to 75 per cent than that in the jugular vein, whereas normally the catalase of the blood of the liver is only about 15 per cent higher than that of the jugular. Hence the ingestion of the meat was stimulating the liver to an increased output of catalase although the blood of the jugular did not indicate it. That was probably due to the rapid destruction of the catalase in the tissues and to the great dilution by the blood. By taking the blood directly from the liver or from a hepatic vein, we have always found a great increase produced in catalase after the ingestion of food.

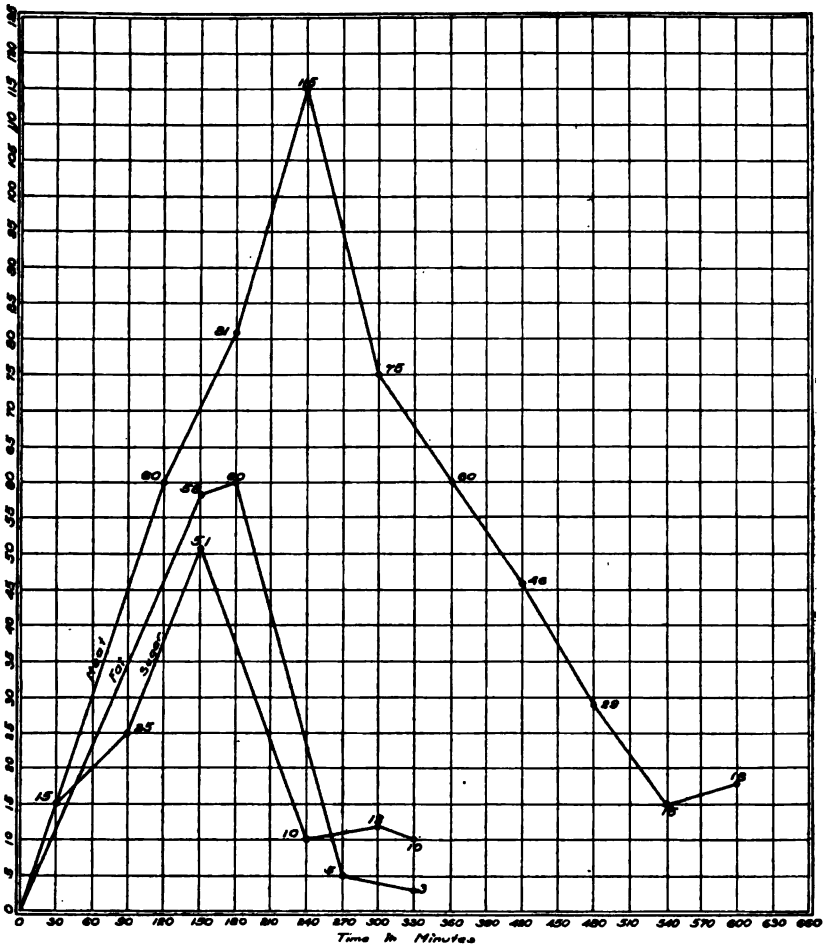

Fig. 2. Curves showing the percentage increase produced in the catalase of the blood by the ingestion of meat, fat and sugar in isodynamic quantities. The figures (0–660) along the abscissa indicate time in minutes; the figures (0–125) along the ordinate indicate percentage increase in catalase.

The last part of this paper is concerned with determining whether the output of catalase from the liver cells continues after the extirpation of the liver, as is the case with the conversion of glycogen into dextrose or the formation of urea from ammonium salts. The animals used were cats and rabbits. After killing the animals the livers were removed and hashed in a hashing machine. The catalase in one gram of the hashed liver was determined according to the method already described.

Curve *A* in figure 3 was constructed from data obtained from determinations of the catalase in one gram of the liver of a cat which was hashed in a hashing machine immediately after its removal from the animal. This hashed material was placed in a glass vessel and covered. A determination of the catalase of one gram of this material was made immediately. After fifteen minutes another determination was made. Similarly, determinations were made after thirty, forty-five, sixty, seventy-five, ninety and one hundred and five minutes. The results of the determinations are given in curve *A*. It will be seen that immediately after the removal and hashing of the liver one gram of the material liberated 820 cc. of oxygen from 250 cc. of hydrogen peroxide. After the material had stood for thirty minutes, one gram liberated 940 cc., after standing one hour, 1060 cc., and after one and one-half hours 1100 cc. From these data it may be seen that the catalase gradually increased in the hashed liver on standing and after two hours it had increased about 34 per cent. This material was kept for about twenty-four hours and it was found that there was no further increase in the catalase content. Curve *B* was constructed from data obtained from a cat in a manner similar to that for curve *A*. Curves *C* and *D* were similarly constructed except the livers of rabbits were used instead of the livers of cats. Similar experiments were carried out on the livers of other rabbits and cats except only a piece of the liver was hashed immediately upon removal from the animal, the other parts of the liver being ground at intervals of thirty minutes immediately before the determinations of the catalase were made. It was found that the catalase increased more rapidly in this material than it did in the livers which were ground immediately upon removal from the animals.

We have shown in this paper that when dextrose reaches the liver after absorption from the stomach and intestines, it stimulates this gland to an increased output of catalase. Claude Bernard (1857) showed that when the liver was removed from an animal the stored

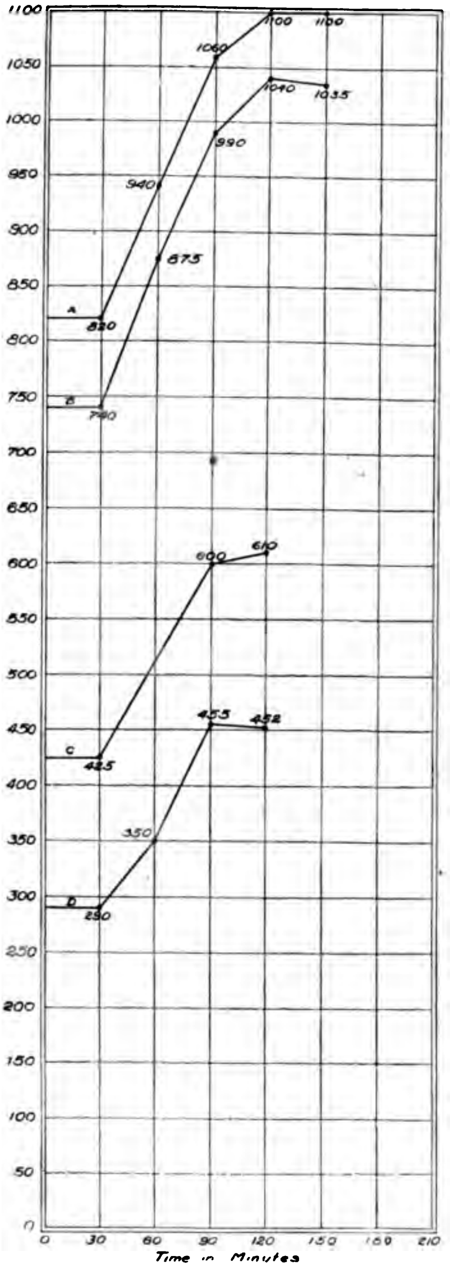

Fig. 3. Curves showing the increase in the catalase of extirpated livers on standing. The figures (0–210) along the abscissa indicate time in minutes; the figures (0–1100) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

glycogen was converted very rapidly into dextrose. The explanation that suggests itself for the increase in the catalase of the liver after its removal from the body of the animal is the stimulation of the cells to an increased output of this enzyme by the dextrose arising from the glycogen. This observation would also seem to offer an explanation for the fact that the blood taken directly from the liver or from one of the hepatic veins contains normally more catalase than the blood from any other part of the body. The preceding experiments would seem to indicate that the dextrose, which is being formed continually from the stored glycogen, serves not only as a source of energy when carried to the muscles and oxidized, but acts also to stimulate, particularly in the liver, an increased output of catalase which brings about oxidation, in some as yet unknown way.

We have already shown that when oxidation was increased, as for example, by increasing the amount of work, by thyroid feeding, by fighting and during the excitement stage of ether anaesthesia, there was an accompanying increase in catalase, due to the stimulation of the liver to an increased output of this enzyme, and that when oxidation was decreased or rendered defective, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, by extirpation of the pancreas, thus producing pancreatic diabetes with resulting defective oxidation, in deep ether anaesthesia and in "shock," there was an accompanying decrease in catalase. In the present paper it is shown that the end products of digestion stimulate the liver, gastric and intestinal glands to an increased output of catalase parallel with the increase produced in oxidation, and in a previous paper it was shown that during starvation there was a decrease in catalase accompanying the decrease in oxidation. We had also found that a great increase was produced in the output of catalase from the liver by stimulating the splanchnic nerves distributed to this organ. Hence it may be assumed that the output of catalase from the different glands, particularly the liver, is normally controlled by nervous as well as chemical stimuli. In combat, for example, it is probable that the increase in catalase with resulting increase in oxidation is brought about by nervous stimuli reaching the liver over the splanchnics, between meals the output of catalase is controlled principally by the dextrose constantly being formed from the glycogen, during and immediately following meals by the end products of digestion absorbed from the stomach and intestines.

SUMMARY

Ingestion of the foodstuffs increases the catalase of the blood and hence of the tissues parallel with the increase in heat production. The increase in catalase is due mainly to the stimulating effect of the absorbed foodstuffs on the liver. The ingestion of protein in keeping with its greater stimulating effect on heat production produces a greater increase in catalase than fat or carbohydrate. After the removal of the liver from the body of an animal, the liver cells continue to liberate catalase for about two hours, due presumably to the stimulating effect of the dextrose formed from the glycogen.

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THE REGULATION OF RENAL ACTIVITY

VII. THE BALANCE BETWEEN THE REGULATION BY ADRENALIN AND BY PITUITRIN

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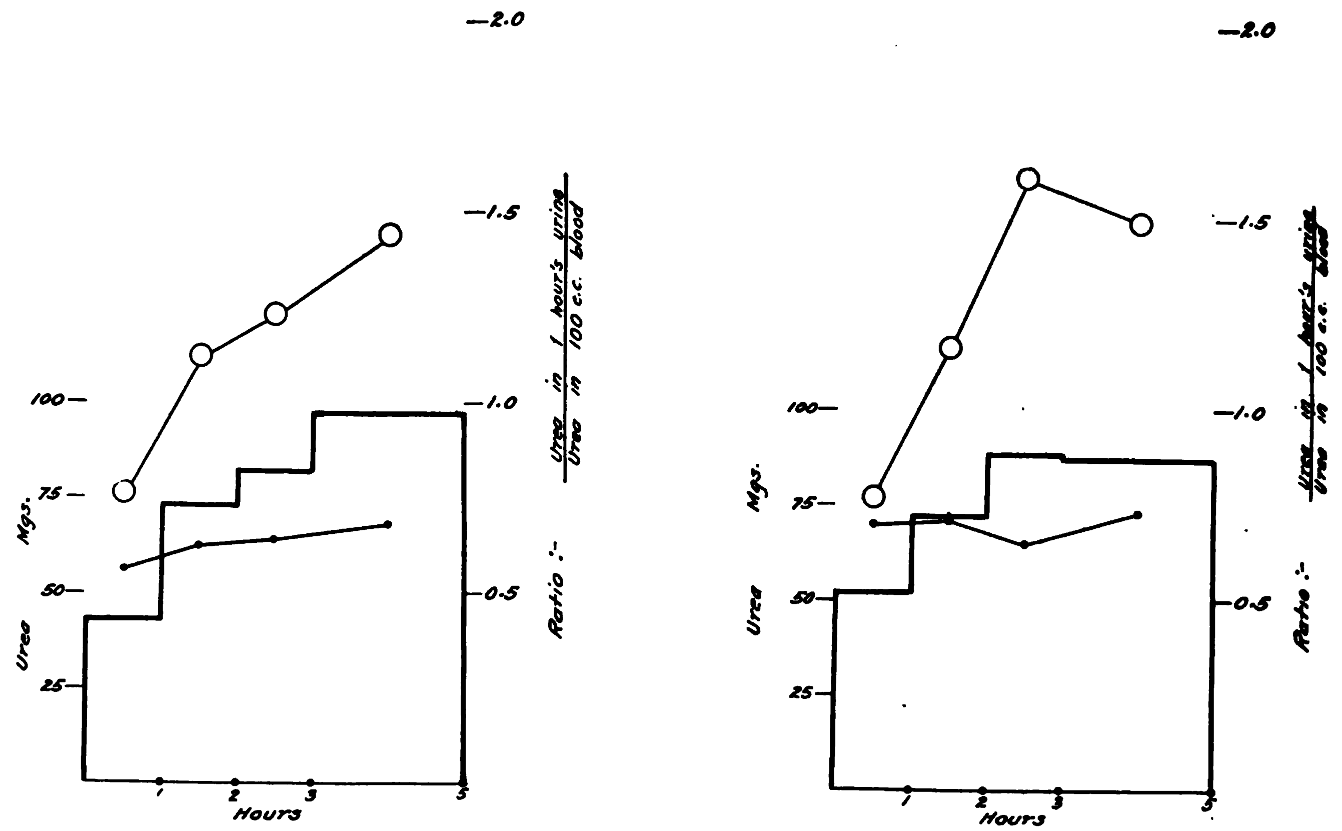
Adrenalin (1) and pituitrin (2) have been shown to regulate the excretion of urea in contrary directions. In this paper experiments are given which show that the injection of amounts of adrenalin which greatly increase the activity of the kidney and of amounts of pituitrin which markedly depress that activity, have no effect when they are given together in a certain balanced proportion. On the other hand, when a mixture of both is injected in which this balance is upset by a preponderance of one or the other, a modified adrenalin or pituitrin

TABLE 1

Comparison of a group of 18 rabbits without and with a mixture of 0.25 cc. of adrenalin and 0.025 cc. pituitrin. No effect on the ratio

PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.25 CC. ADRENALIN AND 0.025 CC. PITUITRIN			PROBABLE DIFFERENCES BETWEEN THE AVERAGE RATIO WITHOUT AND WITH THE MIXTURE OF ADRENALIN AND PITUITRIN	ACTUAL DIFFERENCES BETWEEN THE AVERAGE RATIO WITHOUT AND WITH THE MIXTURE OF ADRENALIN AND PITUITRIN
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	43	56	0.76	52	70	0.77	±0.10	+0.01
II	73	62	1.12	72	71	1.16	±0.12	+0.04
III	82	64	1.23	88	65	1.61	±0.14	+0.38
IV	97	68	1.44	87	73	1.50	±0.14	+0.06



Without adrenalin and pituitrin. With 0.25 cc. adrenalin + 0.025 cc. pituitrin.

Fig. 1. Showing no effect from a balanced mixture of adrenalin and pituitrin.

effect is produced. Adrenalin and pituitrin are thus mutually antagonistic and each may neutralize the effect of the other.

The averages from a group of rabbits under the standard control conditions and after the injection of 0.25 cc. adrenalin alone and of 0.025 cc. pituitrin alone were obtained. A fourth series of experiments was then carried out in which these same quantities of adrenalin and pituitrin were mixed and injected together. The average results of the control and mixture experiments are given in table 1 and figure 1, and the details of the latter in table 5.

TABLE 2

Comparison of a group of 4 rabbits without and with a mixture of 0.25 cc. adrenalin and 0.0125 cc. pituitrin. A modified adrenalin effect on the ratio

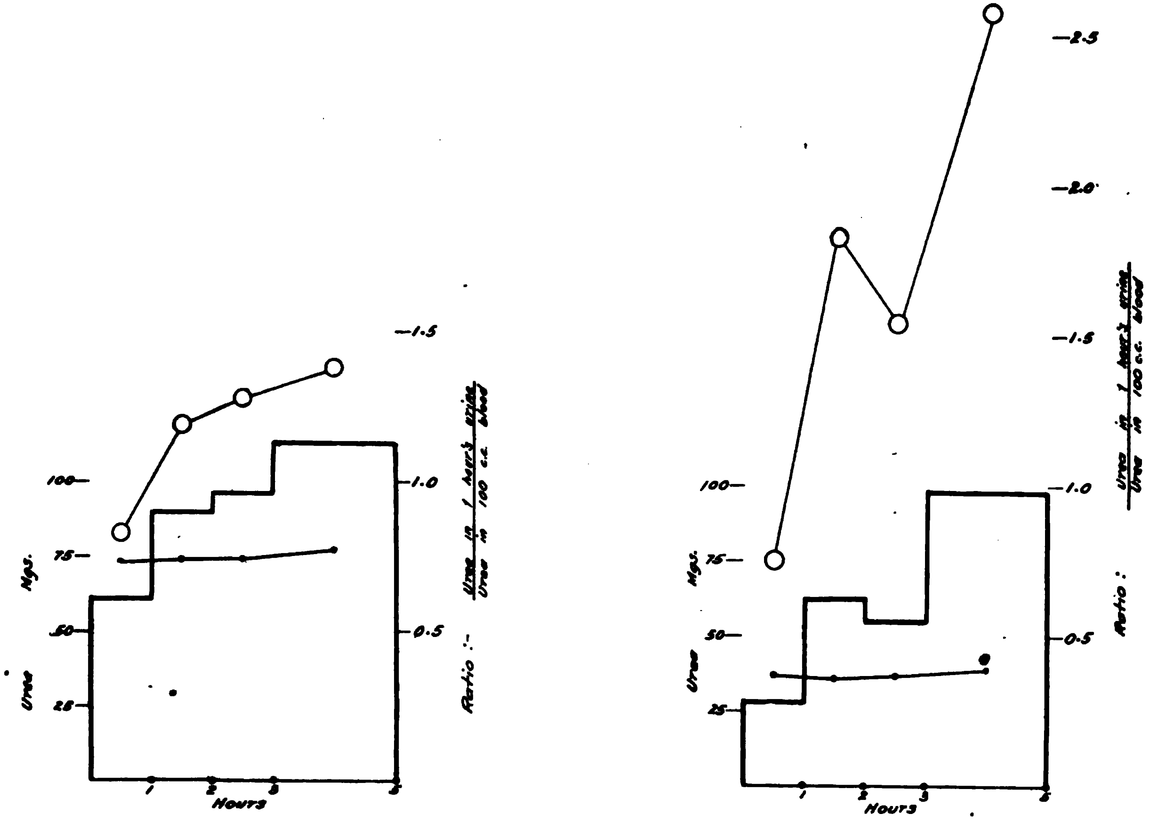
PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.25 cc. ADRENALIN AND 0.0125 cc. PITUITRIN		
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	28	37	0.75
II	90	74	1.19	63	36	1.83
III	96	74	1.28	55	37	1.54
IV	113	77	1.48	98	39	2.58

As compared with the figures for the controls, those of the adrenalin experiments indicated a marked increase in the urea excreting function and those in the pituitrin experiments, a pronounced decrease. But there is no appreciable change in the mode of action of the kidney when both are given together. The stimulating influence of the adrenalin is almost exactly counter balanced by the depressing effect of the pituitrin.

TABLE 3

Comparison of a group of 4 rabbits without and with 0.125 cc. adrenalin and 0.125 cc. pituitrin. A modified pituitrin effect on the ratio

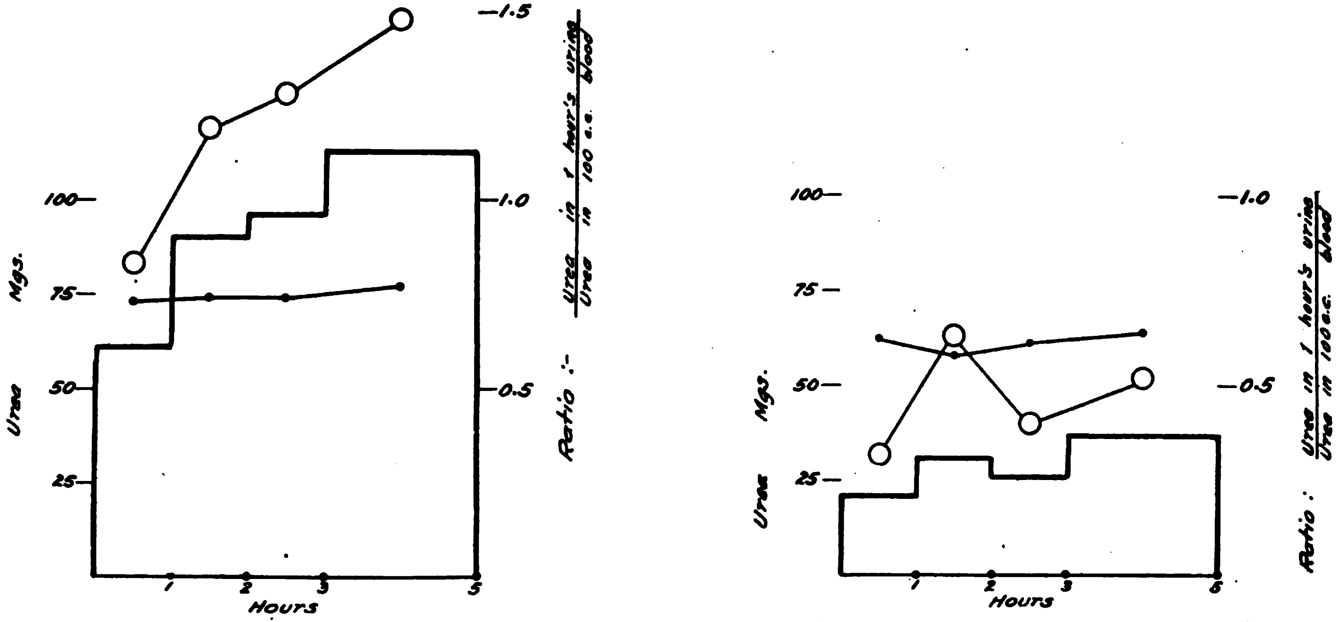
PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.125 cc. ADRENALIN AND 0.125 cc. PITUITRIN		
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	21	62	0.32
II	90	74	1.19	31	58	0.63
III	96	74	1.28	26	61	0.40
IV	113	77	1.48	37	64	0.52



Without adrenalin and pituitrin.

With 0.25 cc. adrenalin + 0.0125 cc. pituitrin.

Fig. 2. A modified adrenalin effect from a mixture of adrenalin and pituitrin in which the adrenalin predominated.



Without adrenalin and pituitrin.

With 0.125 cc. adrenalin + 0.125 cc. pituitrin.

Fig. 3. A modified pituitrin effect from a mixture of adrenalin and pituitrin in which pituitrin predominated.

With a mixture in which the proportion of adrenalin is relatively greater, some increase in the activity of urea excretion is produced though the effect of the pituitrin is still apparent, since the increase is not so great as when the same amount of adrenalin is given alone.

When the proportions are again altered so that the amount of pituitrin is relatively greater than in the balanced mixture, a depression of kidney function follows in which the opposing action of the adrenalin can still be traced.

DISCUSSION

The experiments given in this last paper enlarge the significance of our previous results. For while it is of interest that adrenalin stimulates and that pituitrin depresses the activity of the kidney, the fact that they have effects which are so exactly the reverse of each other that the influence of either may be nullified by the simultaneous administration of a certain quantity of the other, may have a theoretical significance of a higher order. This fact suggests that the resultant of the balance in the blood between the amounts of opposed active principles secreted by the suprarenal and pituitary glands may be a factor in determining the state of renal activity

But there is one aspect of our results from which a deduction of more than hypothetical value may be drawn. Adrenalin and pituitrin, given as we gave them, do not exert their effects on the excretion of urea indirectly through influencing the circulatory conditions in the kidney, but act directly on the urea secreting mechanism. That pituitrin acts through the medium of the nervous system is probable, but with adrenalin there is more than probability, there is a high degree of certainty that it can act only on the termination of sympathetic nerve fibers (3). Since the kidney is supplied with many nerve fibers which end not on the vessels, but on the secreting cells of the glomeruli and tubules, we cannot, in view of the secretory effect of adrenalin, avoid the conclusion that these are secretory nerve endings which it stimulates. And since not only adrenalin, but in all probability pituitrin also, simply accelerate or depress nerve impulses, their effect on the secretory activity of the kidney may be regarded as an argument in favour of a direct regulation and coördination of the activities of the kidney through the central nervous system. We believe that in the case of adrenalin this argument has the force of a proof.

In regard to the hypothesis of an adrenin pituitrin balance in the blood brought to the kidney, we are aware that our experiments only suggest and do not in themselves prove the existence of such a type of physiological regulation. And the history of theories as to the function of the adrenin of the suprarenal glands based on the results of the intravenous administration of adrenalin is a warning against attaching too much importance to experiments in which active principles are injected from without into the body in ways which can never exactly duplicate the rate and manner in which they are distributed to the tissues from the sites of their formation within the body. Yet we feel that experiments such as ours in which the gland extracts are administered so that they gain entrance to the body only slowly, in minute quantities and over a considerable period of time, should carry more weight in deciding questions of physiological, as opposed to pharmacological, action, than those in which extracts are suddenly injected into the circulation in amounts which, for a short time at least, must induce concentrations of these substances in the blood out of all proportion higher than any which can ever have previously existed there. This latter is a grossly artificial method which certainly bears no relation to physiological processes, while the former is at least more nearly comparable to what we know of the normal modes of entrance of these secretions into the blood stream.

There is one comparatively simple way in which the hypothesis of an adrenin pituitrin balance may be tested. If the suprarenal glands are removed, the pituitary secretion will be left unopposed and there should be a depression of renal activity. Similarly, when the pituitary gland is removed, the suprarenal secretion will be unopposed and the activity of the kidney should be increased.

We had not much more than started this phase of the work when other duties required our attention for an indefinite period of time. We had compared the effect on kidney function of operations in which the suprarenals were exposed, or one suprarenal removed, with the effect of double suprarenalectomy. We found that when both glands were removed, the function of the kidneys was depressed to a much greater degree than after other procedures in which the factors of trauma and anaesthetic were as far as possible the same. The decrease in function was indeed in most cases so marked that little more than traces of urea were excreted and the value of the ratios approached zero. As a consequence the percentage of error of the determination was considerable and these preliminary experiments, though they established the general fact of a great loss of capacity for urea excretion, were not sufficiently accurate to allow us to settle the point in which we were especially interested, i.e., the form of the ratio-curve in the absence of the suprarenals. We can, however, fully agree with Marshall and Davis' (4) conclusion that the decrease in the rate of excretion of urea, chlorides and creatinin which they observed in cats after double suprarenalectomy may be regarded as evidence of a decrease in the activity of the kidney.

We had not even begun to attempt operations on the pituitary gland. It is stated by Cushing (5) that in dogs the volume of urine is three to five times greater after the removal of the posterior lobe of the pituitary gland, and that this diuresis persisted for some time. It is interesting to note, however, that it was not a permanent condition. In this connection the results of one of our experiments in which a rabbit survived the removal of both suprarenals may be worth quoting. The preliminary control experiment under standard conditions gave an average ratio of 1.54. Five days later both suprarenals were exposed and manipulated through extraperitoneal incisions. Immediately after the animal had recovered from the anaesthetic the experiment was commenced. The average ratio was 2.13, which was exceptional in that such an operation usually reduced the activity of the kidney. One week later the wounds were reopened and both suprarenal glands removed. Ratios were measured immediately afterwards and an average of 0.36 obtained. Though this is a considerable reduction, it is a much higher ratio than we usually obtained after taking out both glands. Next day the rabbit was still alive and the average ratio was found to be 0.68. On the third day after the double suprarenalectomy the ratio had risen to 1.15 and on the seventh day to 1.59. A single experiment is of course of no great value, but it is suggestive when taken in conjunction with the apparently similar experience when the posterior lobe of the pituitary is removed. The simplest explanation, no doubt, is that remaining pituitary or suprarenal tissue, though at first insufficient, was later able to right the balance; but it is also possible that the nervous system may in time accommodate itself to the absence of either pituitrin or of adrenin, so that the equilibrium between those nervous impulses which accelerate and those which depress the activities of the kidney is again reëstablished.

Beyond giving our reasons for concluding that adrenalin and pituitrin, when administered subcutaneously, cannot influence the secretion of urea by virtue of any effect on the circulation of blood through the kidney, we have so far avoided any reference to the rate of blood flow as a factor in determining renal activity. It is, technically, a very difficult matter to obtain valid data on the relation between blood flow and variations in the secretion of the normal kidney. Stromuhr measurements necessitate a degree of interference with the nervous connections of the kidney which is in itself amply sufficient to cause large deviations from normal blood flow and normal function. But we had hoped we might be able to obtain evidence in regard to this question by a method which does not require the attachment of any mechanical measuring device to the renal vein. By using a syringe with a fine needle it is possible to obtain at intervals samples of blood from the renal vein so that the urea concentration of the blood as it leaves the kidney can be determined. At the same time blood may be taken from the jugular vein as representing the blood of the renal artery, so far as its urea concentration is concerned. If the rate of flow of urine and the rate of urea excretion from the same kidney are measured over the period during which these blood samples are being collected, all the data are at hand for the calculation of the rate of blood flow through the organ. But in rabbits even this relatively minor degree of manipulation was sufficient, in our hands at least, to cause a cessation of the flow of urine. In cats under the influence of powerful diuretics measurements were made, but we cannot place any reliance on them from the point of view of either normal blood flow or normal renal function.

An endeavor was later made to obtain blood from the renal vein so quickly that the decrease in its urea content as compared with that of the general circulation, might be taken as indicating the degree to which the urea excreting activity of the undisturbed kidney had reduced the urea concentration of the blood during its passage through the renal tissues. After a little practice it was found that after a wide and rapid incision through the flank, the left renal vein could be snipped with scissors and a sample of blood collected within a very short space of time, and without touching the kidney. In seventeen of these experiments (6) less urea was found in the blood of the renal vein than in the blood of the renal artery or jugular vein, but the average decrease amounted to only 7.4 per cent of the urea concentration of the general blood stream. Since then the kidney received a much larger amount of urea than it eliminated, it would seem probable that physiological variations in the volume of blood passing through it would have little if any effect on the rate of urea excretion. This argument, of course, is not conclusive since we can never be sure that operative procedures leave kidney function undisturbed, however quickly they are carried out. Yet it is not negligible when direct evidence is so difficult to secure.

It is an undisputed fact that an extreme reduction in the blood supply to the kidney is followed by a cessation of function. This effect, however, is more probably due to a reduction in oxygen supply than to a decrease in the amount of urinary constituents brought to the kidney. But the oxygen concentration in the blood may be markedly reduced without interfering with renal function. We had commenced observations on the urea secreting capacity under strain in rabbits rendered anemic by the withdrawal of large amounts of blood while the function was being measured. We hope at some future date to be able to continue these experiments, but so far as we went we did not find any significant alteration in the ratio even when the hemoglobin percentage fell below 40 per cent. We believe, therefore, that the kidney is supplied with an excess of both urea and oxygen and that such variations in the total amounts of these substances passing through the kidney as occur with changes in the rate of blood flow through it will not appreciably alter the state of its functional activity, so long as these amounts exceed a certain critical minimum. We regard, then, the total volume of blood supplied to the kidney over any given period as a factor analogous to the total mass of renal tissue (7). Both are ordinarily merely potential factors which become operative as direct determinants of function only under exceptional and extreme conditions.

There remains the question as to the bearing of the theory of a direct regulation of renal secretion through the nervous system and of the subsidiary hypothesis of an adrenin pituitrin balance, on the observed facts in regard to kidney function which were detailed in the first three papers of this series.

Certain of these phenomena—the variation in rates measured at the same blood urea concentration, the increase in the activity of the kidney under increasing strain and the decrease in variability with increase in strain—were observed in man as well as in the rabbit. No discussion is required to show that they are just such phenomena as might be expected to occur in the function of any organ under the influence of the nervous system. Their relation to the hypothesis of an adrenin pituitrin balance in the blood has been dealt with elsewhere (8).

But there are still two characteristics of normal kidney function, one peculiar to the rabbit—the gradual increase in the urea excreting capacity during successive observations (9)—and the other the relationship between water administration and states of activity in urea excretion, which we have noted but have not hitherto attempted to explain.

There was nothing in the observations we carried out on man to lead us to expect any increase in the rate of urea excretion in consecutive observations on rabbits whose blood concentration remained constant, and no explanation suggested itself until we had commenced the work with adrenalin. It then occurred to us that a difference between the conditions of our experiments on man and on the rabbit may have been responsible for this striking divergence in the behavior of the kidney. In man there was no catheterization and by using sharp needles even minor degrees of discomfort were avoided in obtaining the blood. The subjects were instructors and students who were in no anxiety at the prospect of being bled. In the rabbits, on the other hand, there was a series of nine manipulations over a period of five hours commencing with the passage of the stomach tube, against which they usually struggled violently, and often involving, in the catheterizations, a certain amount of trauma on account of the compression over the bladder and the repeated rotations and partial withdrawals and reintroductions of the catheter, employed in the endeavor to make sure that the urine or wash water had been removed as completely as possible. These differences in the external conditions in the experiments on man and on the rabbit induced a difference in the internal conditions also, for while in the one case the subjects were undisturbed, in the other they were much excited.

The influence of excitement on the rate of secretion of adrenalin is still under discussion, for Stewart and Rogoff (10) have not found the increase which Cannon and others (11) observed. But some recent measurements of Bedford (12) happen to parallel closely the time interval relations of our experiments. He produced shock in anaesthetized animals and determined the concentration of adrenalin in the blood of the suprarenal vein in successive samples drawn at intervals over a period of several hours. It was found that the concentration of adrenalin did not at once rise to a high level but only gradually increased so that the maximum was not reached for several hours. The increase, though slow, was pronounced. Thus in one case there was over thirty times more adrenalin at the end than at the commencement of the experiment. Now it will be remembered that the increase in the activity of the kidney in our experiments is also gradual and does not approach its highest point until after three or four hours.

When this concordance between the time required for the suprarenal glands to markedly increase their adrenin putput and the time at which the rabbit's kidney shows its greatest activity during a period of continued excitement, is taken in conjunction with the fact that the subcutaneous injection of adrenalin accentuates the degree of increase in activity but does not otherwise alter the mode of action of the kidney, there seems to be reason for the supposition that this increased activity of the kidney of the rabbit under these conditions is associated with and is intensified by an increase in the adrenin content of the blood arising under the influence of the physiological stimulus of excitement.

The only other observation whose bearing on our hypothesis remains to be discussed, is the relation between water administration and renal activity in the excretion of urea. We have not given any details of the influence of adrenalin and of pituitrin on the excretion of water, partly because it has been already described by others, (13), (14), but mainly because we have not the data which are necessary to decide whether or not the changes they induce in water excretion arise from alterations in renal activity. It is evident that the mere observation that the output of a urinary constituent is altered by adrenalin and pituitrin does not in itself prove that any change in the activity of the kidney has occurred. For the kidney may have maintained its accustomed mode of reaction and the altered output be the result of its passive adjustment to changes in the concentration of that constituent in the blood. It is only for the urea excreting function that we have excluded this possibility. Yet we believe it is highly probable that it will be found that all the various activities of the kidney are stimulated by adrenalin and depressed by pituitrin and that the increase in the volume of urine after adrenalin and its decrease after pituitrin is, in the main at least, caused by alterations in renal activity and not by changes in the water content of the blood. We refer to this question now because in studying the effect of the administration of varying amounts of water on urea excretion, we observed a curious divergence in its influence under different conditions.

Our first experiments on the effect of water were carried out with a view to determining whether the changed action of the kidney in excreting urea after adrenalin and pituitrin might not be a secondary result of the concomitant change in urine volume. But when we gave 100 cc. of water so that the volume of urine was increased to a degree approximating that obtained after adrenalin, there was no increase in the urea excreting function nor any appreciable decrease when the volume of urine was lowered to amounts equivalent to those obtained after pituitrin by the administration of magnesium sulphate by mouth. This early result indicated that urea and water effects were independent and forecast our later conclusion that there was no causal relation between variations in urine volume and variations in renal capacity for urea excretion. In this particular instance, therefore, in which moderate quantities of water were given, there was no accompanying change in the activity of the kidney for urea excretion.

We have elsewhere (15) referred to other experiments, carried out on man, in which the drinking of very large quantities of water was associated with a constant acceleration of the work of the kidney in excreting urea. In these experiments the subject drank 1000 cc. of water and thereafter every quarter or half hour amounts of water equivalent to the volume of urine excreted. In this way the water excreted was continually returned so that the water content of the body was for a period of several hours kept much higher than it normally would have been. The rate of water excretion gradually increased until enormous quantities were being eliminated, amounting in some cases to a 24 hour rate of over 20,000 cc. Under these conditions, there was a very definite increase in the rate of urea excretion, though there was no significant change in the level of the blood urea concentration. We therefore concluded that in this instance, in which very large quantities of water had been given, some unknown factor had produced an increase in the urea excreting activity of the kidney. The nature of this factor was left undetermined but we decided it was not the increased water content of the blood because there was no constant relation between the amounts of water eliminated and the degree of increase over the normal in the rate of urea excretion.

The third instance in which the giving of water was accompanied by the reverse effect, i.e., by a decrease in urea excreting activity, was found in some experiments on rabbits. A double set of seventeen observations were made on a group of ten rabbits, with and without the administration of 25 cc. of water by stomach tube. The averages are given in table 4 and charted in figure 4.

We were at first glance inclined to think that the decrease in the ratios in the water experiments was due to chance, for the actual differences are not great. But they are in the same direction in each period and calculation from the probable differences of the averages of the ratios shows that there is only about one chance in one hundred and seventy that this was a chance variation. We were therefore

TABLE 4

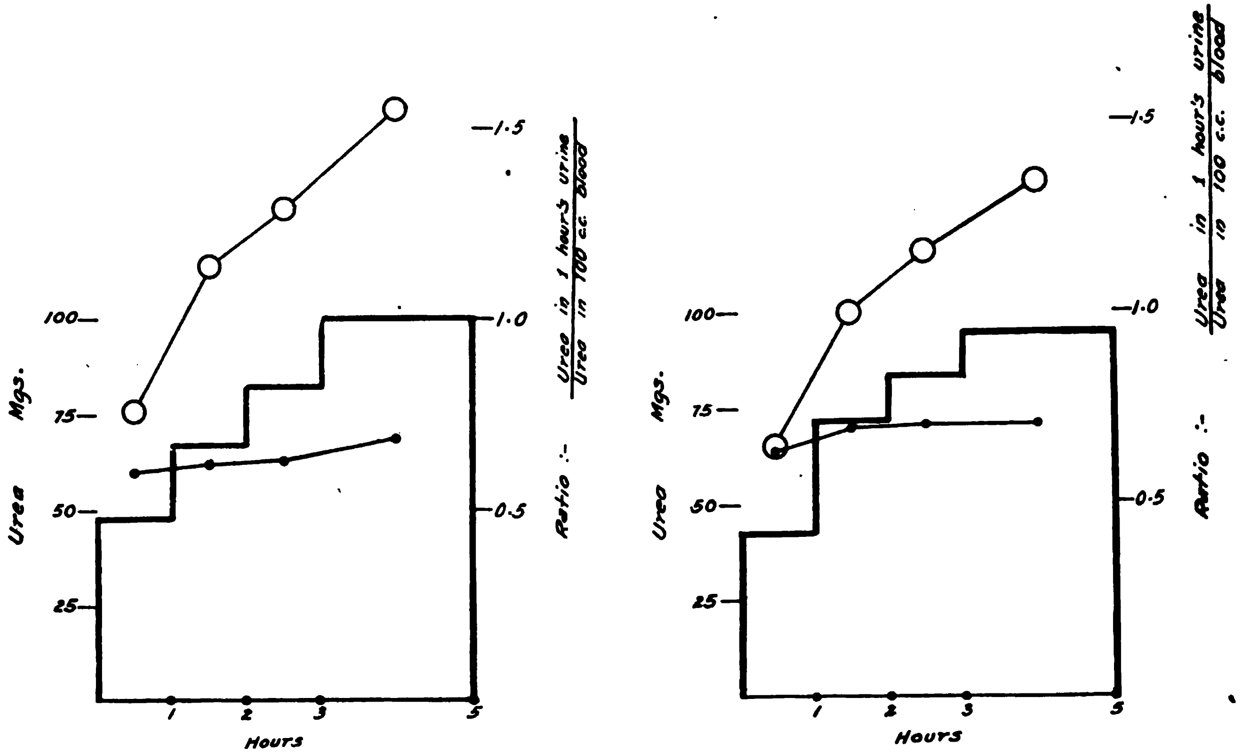
Comparison of averages from 17 experiments on a group of 10 rabbits without and with 25 cc. H_2O

PERIOD	WITHOUT WATER			WITH 25 CC. WATER			PROBABLE DIFFERENCES BETWEEN THE AVERAGE RATIOS WITHOUT AND WITH WATER	ACTUAL DIFFERENCES BETWEEN THE AVERAGE RATIOS WITHOUT AND WITH WATER
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	48	60	0.76	43	64	0.65	±0.068	−0.11
II	67	62	1.14	72	70	1.00	±0.075	−0.14
III	82	63	1.29	84	71	1.16	±0.097	−0.13
IV	100	69	1.55	95	71	1.34	±0.106	−0.21

TABLE 5

A mixture of 0.25 cc. adrenalin and 0.025 cc. pituitrin

	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
Rabbit No.	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio
85	3	38	0.00	13	38	0.35	29	44	0.67	55	40	1.38
86	lost	lost	lost	31	42	0.73	75	42	1.78	73	43	1.69
88	43	72	0.59	82	72	1.14	88	73	1.21	127	75	1.69
96	84	66	1.27	104	66	1.57	133	69	1.93	132	65	2.02
97	86	51	1.69	93	48	1.93	94	45	2.08	92	47	1.95
98	28	39	0.70	46	39	1.19	99	33	3.00	76	32	2.38
100	2	105	0.00	8	111	0.07	7	113	0.06	5	118	0.05
93	58	58	1.21	74	45	1.63	99	45	2.20	109	51	2.14
65	14	34	0.26	67	54	1.24	91	56	1.64	89	54	1.64
66	9	32	0.28	33	39	0.85	25	33	0.77	12	60	0.20
67	17	54	0.31	36	56	0.64	33	56	0.58	61	60	1.01
68	75	54	1.38	104	60	1.74	106	68	1.56	116	72	1.61
71	27	54	0.50	50	54	0.93	66	57	1.16	64	62	1.03
73	55	57	0.96	128	46	2.78	119	50	2.38	75	53	1.41
90	16	21	0.77	12	19	0.62	63	24	2.63	88	26	3.39
99	62	72	0.86	93	97	0.96	104	69	1.50	104	78	3.33
103	157	154	1.02	123	144	0.85	197	138	1.43	139	145	0.96
105	85	134	0.64	117	153	0.76	lost	lost	lost	94	149	0.63
Average.....	52	70	0.77	72	71	1.16	88	65	1.61	87	73	1.50



Without water.

With 25 cc. water.

Fig. 4. Showing a slight depression of renal activity following the administration of water.

forced to conclude that the giving of relatively small amounts of water to rabbits was accompanied by a decrease in the urea excreting activity of the kidney due to the operation of some unknown factor. In our view the reason for both the increase in kidney activity after water administration in man and the decrease which occurred in the experiments on the rabbit is to be found in a consideration of the divergent effect of the water, not on the kidney alone but on the body as a whole.

In the experiments on man we continually nullified the work of the kidney by pouring back the water which it had eliminated. We thus tended to produce an artificial enrichment of the organism with water and the kidney made greater and ever greater efforts in preventing a harmful disturbance in the osmotic equilibrium of the tissues. We believe that the diuresis which occurred was greater than could be accounted for mechanically through the increase in the free water of the blood, and that the unknown factor which stimulated the kidney to these more than wonted exertions was the accelerating influence of nerve impulses aided by an increase in the relative proportion of the adrenin as compared with the pituitrin of the blood. The indication of the operation of this factor is the increased activity in the excretion of urea.

In the experiments on the rabbit no excessive amount of water was given. A quantity of 25 cc. no doubt seems a large amount for a rabbit, and in proportion to the body weight may be regarded as equivalent to about 750 cc. for a man. But quantities are large or small only in relation to the requirements of the tissues, and in these rabbits the need of the body for water was great since they had received no food or water for at least seventeen hours before the experiment began. But this water introduced into the gastro-intestinal tract could only reach the tissues through the blood stream and in doing so would increase the concentration of free water in the blood. The unregulated kidney must automatically respond to this stimulus and an undue proportion of the needed water would have been lost to the body. We believe, therefore, that when food and water are withheld there is a purposeful adaption of kidney function through a relative increase in the pituitrin as compared with the adrenin content of the blood, and that the decrease in the activity of the urea excreting function is only one example of a general state existing in all the departments of renal function. This regulation is, of course, not peculiar to the rabbit. We have elsewhere cited instances of the decrease in function in man which occurs after periods of abstention from food and water (16).

These explanations of our results have only a hypothetical value. For we have not demonstrated any change in the adrenin pituitrin balance, and we have only assumed that the excretion of water resembles the excretion of urea in varying in a manner which cannot be fully accounted for by changes in the water content of the blood. Until some method is devised by which the adrenin and pituitrin content of the blood reaching the kidney can be determined, and until the water in the blood available for excretion can be measured, these assumptions must remain untested by experiment

The question of the exact mechanism whereby the kidney is regulated is therefore not definitely decided by these experiments. We should indeed be quite prepared to find that changes in the adrenin-pituitrin balance are only operative in times of emergency as adjuvants to the direct nervous control. The fact that all the nerves going to the kidney may be cut and that the kidney may even be transplanted on to the splenic vessels and yet continue to function "normally" (17) does not exclude this possibility. Our criterions of normality are still too vague.

The question which *is* decided is that there is a regulation in addition to, and distinct from, the mechanical regulation arising from the passive adjustment of the kidney to changes in the concentration of urinary constituents in the blood. And still more important is the demonstration that this higher regulation may annul or overrule the influence of physical and chemical factors in the environment of the kidney, in order that the requirements of the body as a whole may be met. The kidney can not be regarded as an isolated mechanism. It is coördinated in those active adaptions which continuously maintain the harmonious equilibrium of the living organism.

CONCLUSIONS

1. The subcutaneous injection of amounts of adrenalin which increase the urea excreting activity of the kidney, and of amounts of pituitrin which depress that activity, have no effect when they are injected together in a certain balanced proportion.

2. All grades of stimulation or depression may be induced by the injection of mixtures of adrenalin and pituitrin in which this balance is deflected by a preponderance of one or the other.

3. In the rabbit the removal of both suprarenal glands is followed by a depression of the urea excreting activity of the kidney, which is

greater than that which follows similar operations in which the suprarenals are not removed.

4. The facts in regard to urea excretion given in these and other papers are reviewed and from them the conclusion is reached that under physiological conditions the urea excreting activity of the kidney is determined by two main factors. There is a fixed and mechanical regulation through the urea concentration of the blood but there is also another and overruling type of regulation which acts through the medium of the central nervous system.

5. The mode of action of the regulation through the nervous system is discussed and it is suggested that variations in the balance between the rates of secretion of active principles from the suprarenal and pituitary glands may play a part in the mechanism through which it acts.

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THE EFFECT OF HOLDING THE BREATH AND OF REBREATHING ON THE RISE OF CO_2 TENSION IN THE LUNGS, AND THE DETERMINATION OF THE CO_2 TENSION OF THE "VENOUS PULMONARY AIR"

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INTRODUCTION

A method has been recently suggested by Henderson and Prince (1) for the determination of the CO_2 tension of the "venous pulmonary air" and attention called to the importance of this determination. Christiansen, Douglas and Haldane (2) have also described a method for the determination of this function based on the use of the lungs as an aerotonometer. They found that the CO_2 pressure in the mixed venous air could not be obtained by holding the breath for the reason that the alveolar CO_2 pressure continues to rise with the length of time that the breath is held, there being no pause to indicate when the CO_2 in the alveolar air is in equilibrium with that of the venous blood. Their method is briefly as follows: After a maximal expiration a maximal inspiration of a mixture of CO_2 and air is made and the gas mixture held in the lungs for a few seconds, and then exhaled and an alveolar sample analyzed for CO_2. After a further interval another, and sometimes a third, alveolar sample is taken and analyzed. From the increase or decrease of CO_2 in the mixture after inhalation the venous CO_2 pressure is inferred, after correction for the influence of oxygenation in the lungs.

Boothby and Sandiford (3) also used the aerotonometric method to determine the CO_2 tension in the venous blood. Their method is a modified form of that of Christiansen, Douglas and Haldane. After a mixture of CO_2, air and O_2 is inspired, it is held for about five seconds and then an expiration to the mid-level made for the first alveolar sample, after which the breath is held for as long as possible and a maximal expiration made and the second alveolar sample taken.

In an earlier paper Boothby (4) calculated the venous CO_2 tension by the nitrous oxide method, from the consumption of O_2, the flow of blood through the lungs, the respiratory quotient and the percentage saturation of the Hb.

Wardlaw (5) has recently studied in detail the rise in CO_2 tension of the air in the lungs when the breath was held for various periods of time and when the air was rebreathed from a bag. In the case of holding the breath he found that as the length of time increased the alveolar CO_2 tension rose at a continually decreasing rate for about 30 seconds, the alveolar tension being an exponential function of the period for which the breath was held, and that when the breath was held for a long enough period the CO_2 tension gave indications of attaining a certain fixed value, (48.5 mm. Hg.).

When the same air was rebreathed Wardlaw found again that the alveolar CO_2 tension rose at a continually decreasing rate, reaching a final value of about 56.5 mm. Hg. During the first five seconds the CO_2 tensions rose by practically the same amount as when the breath was held, but between the 25th and 30th seconds the rise was about three times as great as when the breath was held. Furthermore, the total rise in the alveolar CO_2 tension in 35 seconds was nearly 40 per cent greater than the rise occurring in the same period when the breath was simply held, the alveolar tension being again an exponential function of the period for which the contents of the lungs are rebreathed.

The rate of gaseous exchange in the alveolar air is therefore about twice as great when the movements of breathing are performed as when the breath is held under normal pressure. The cause of this greater increase Wardlaw does not believe to be due to a slowing up of the circulation during the time that the breath is held, for by comparing the effect on the gaseous exchange of one respiratory movement in 20 seconds and of three in the same period with that occurring when the breath is held, he found that the increase in CO_2 was the same whether one or three movements were made. Also he was led to conclude that the increase in the respiratory exchange in a given time was independent of the extent of the respiratory movements; for he again obtained the same increase when four respiratory efforts with the pharynx closed were made as when one or three normal respiratory movements were made in the same period.

Wardlaw also concluded from experimental evidence that holding the breath under increased pressure does not affect the gaseous exchange. On the other hand, holding the breath under negative pressures he found to increase it to such an extent that when the breath was held under pressures of more than −10 mm. Hg. the same acceleration in the rate of the gaseous exchange took place as when the air was rebreathed from a closed bag. He therefore concludes that the respiratory exchange is accelerated during breathing owing to the existence of negative pressure in the chest during the act of inspiration.

It is difficult to reconcile this conclusion with the results of the comparison of the rate of gaseous exchange made by Wardlaw when one and three respiratory movements are made in 20 seconds; for, if by one respiratory movement is meant an inspiration lasting for 20 seconds (which is improbable) then, if the increase in the gaseous exchange is due to the negative pressure, the CO_2 tension should be greater than when three respiratory movements are made in 20 seconds, since the time during which a negative pressure exists is shorter in the latter case. If by one respiratory movement in 20 seconds is meant an inspiration lasting 10 seconds and an expiration lasting 10 seconds, than a negative pressure will exist for an equal length of time in the two cases. But if by one respiratory movement is meant an inspiration of usual duration, followed, after 20 seconds, during which time the breath is simply held, by an expiration, than a negative pressure would exist in the lungs a shorter length of time than when three respiratory movements are made in the same period. This would also be true when the respiratory movements were made with the pharynx closed, since during the time that the expiratory efforts were being made the pressure would be positive, and therefore the rate of exchange would be slowed up, as compared with the rate during the time that the pressure was negative, that is, during the actual inspiration.

THE RISE OF CO_2 TENSION IN THE LUNGS IN HELD AND REBREATHED AIR

In connection with the study of the circulation rate it was suggested to me by Prof. Yandell Henderson that it would be well to make a detailed study of the changes in the CO_2 tension of held and rebreathed air along the lines followed by Wardlaw, and to check up the determination of the venous CO_2 tension by the method suggested by Henderson and Prince.

This most simple method of determining the venous CO_2 tension consists briefly in intermittent, as contrasted with continuous, rebreathing. After a sudden inspiration a quick and deep expiration is made into a rubber bag and the CO_2 tension of this "mixed air" determined by analysis. After a sufficiently long interval to allow the respiration and circulation to return to normal, the lungs are emptied as far as possible and the contents of the bag inhaled, the breath held for a few seconds and the air then exhaled deeply into the bag and its CO_2 tension again determined. This intermittent rebreathing is continued until after successive rebreathings a constant CO_2 tension is found upon analysis. This takes place usually, when the subject is at rest, after the fifth to the sixth inhalation.

In all of the experiments which are described below, on holding the breath and rebreathing air this method of Henderson and Prince was employed to obtain the first gas mixture wherewith to begin. In this way an experimental starting point was obtained in that, by analysis of the expired air, a known CO_2 tension was begun with. Also the percentage of increase can then be calculated. The CO_2 tension of the air obtained in this way was found to vary between 3.25 and 4 per cent.

The greater increase described by Wardlaw in CO_2 tension when air is rebreathed as compared with that obtained when air is held in the lungs has been corroborated. The increase in the CO_2 tension when the breath is held is shown in table 1 and figure 1. The tension of the CO_2 continues to increase with the length of time that the breath is held, but at a decreasing rate, until an apparent maximum is reached as indicated by the percentage of increase. It is impossible for me to hold my breath, under the conditions of the experiment, for longer than about 50 seconds.

Results obtained when air is rebreathed are shown in table 2 and figure 1. Here again the CO_2 pressure continues to rise at a decreasing rate with the length of time that the air is rebreathed and attains also an apparent maximum. The rate of increase, however, is more rapid and the final tension attained higher when the air is rebreathed than when it is held. It is impossible for me to continue rebreathing, under the conditions of the experiment, for longer than 72 seconds, (12 rebreathings). The maximum CO_2 tension seems to be approximately attained after the 9th rebreathing (54 seconds).

This comparison therefore between the rate of CO_2 tension increase and the final tension attained after holding the breath and rebreathing the same air, gives results which are essentially similar to those of Wardlaw. When the air is rebreathed the tension of CO_2 rises more rapidly and attains a higher value than when the breath is held. After

30 seconds for example, there is an average percentage increase of 178 when the air is rebreathed as compared with one of 162 when the breath is merely held.

TABLE 1

The influence of holding the breath on the CO_2 tension

MINUTES HELD	PERCENTAGE OF CO_2								
	Before holding	After holding	Per cent of increase	Before holding	After holding	Per cent of increase	Before holding	After holding	Per cent of increase
5	3.31	4.07	123	3.65	4.34	119	3.47	4.48	129
10	3.43	4.72	138	3.42	4.55	133	3.56	4.98	140
15	3.51	5.33	149	3.54	4.78	135	3.36	4.91	146
20	3.50	5.39	154	3.45	5.14	149	3.61	5.67	157
25	3.25	5.10	157	3.43	5.45	159	3.65	5.95	163
30	3.34	5.34	160	3.52	5.74	163	3.54	5.79	164
35	3.49	5.72	164	3.53	5.79	164	3.67	6.13	167
40	3.57	5.96	167	3.58	6.05	169	3.42	5.95	174
45	3.70	6.40	173	3.58	6.12	171	3.35	5.80	173
50	3.77	6.41	170	3.67	6.39	174	3.49	6.11	175

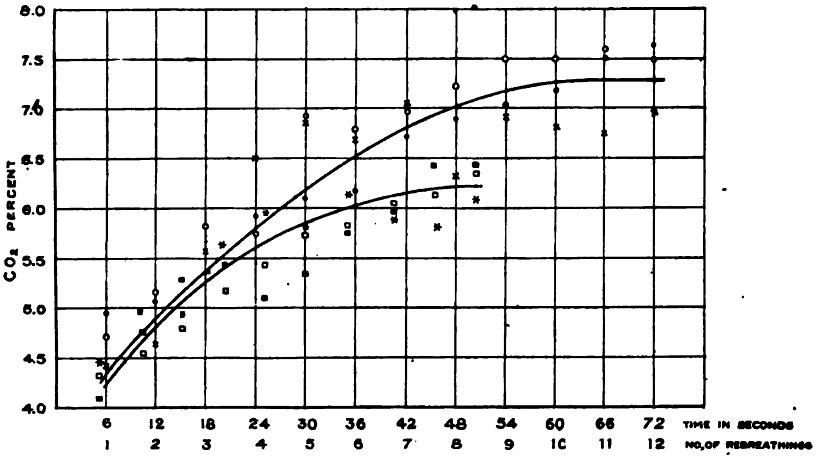

Fig. 1. The influence of holding the breath (lower curve) and of rebreathing (upper curve) on the rise of CO_2 tension in the lungs.

A series of experiments, along the same lines that Wardlaw followed was next made to determine the cause of the greater and more rapid increase in CO_2 tension when the air is rebreathed than when it is held. This involved in the first place the investigation of the effect of varying the number of respirations in unit time.

Table 3 shows the results obtained for 10, 20 and 30 seconds. In general it may be said that increasing the number of respirations in unit time increases the tension of CO_2 in the rebreathed air. In the case of 10 seconds this fact does not come out so clearly. But another significant fact, which will be referred to later, (see tables 6 and 7), is noted; this is that holding the breath for 10 seconds and inspiring it for 5 seconds and expiring it for 5 seconds results in about the same percentage increase of CO_2. This is also seen in the case of 20 seconds, where inspiring for 10 seconds and expiring for 10 seconds results in about the same increase as holding the breath for 20 seconds.

TABLE 2

The influence of rebreathing on the CO_2 tension

MINUTES REBREATHED AND NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2								
	Before	After	Per cent of increase	Before	After	Per cent of increase	Before	After	Per cent of increase
6 (1)	3.69	4.94	134	3.67	4.66	127	3.40	4.45	131
12 (2)	3.59	5.10	142	3.51	5.19	148	3.31	4.63	140
18 (3)	3.35	5.39	161	3.69	5.83	158	3.52	5.63	160
24 (4)	3.39	5.90	174	3.50	5.74	164	3.86	6.52	169
30 (5)	3.40	6.12	180	3.86	6.87	178	3.93	6.88	175
36 (6)	3.30	6.14	186	3.64	6.55	180	3.75	6.71	179
42 (7)	3.45	6.73	195	3.78	6.99	185	3.86	7.03	182
48 (8)	3.50	6.90	197	3.62	7.17	198	3.46	6.32	185
54 (9)	3.41	7.02	206	3.77	7.50	199	3.67	6.94	189
60 (10)	3.57	7.21	202	3.75	7.50	200	3.54	6.83	193
66 (11)	3.56	7.55	212	3.71	7.61	205	3.49	6.75	194
72 (12)	3.69	7.68	208	3.67	7.52	205	3.63	6.97	192

Wardlaw obtained the same percentage of CO_2 increase over that found after holding the breath, when one respiratory movement in 20 seconds was made as when three were made. My results do not agree with this. In the first place, one respiratory movement (in 10 or 20 seconds) in which the inspiratory and expiratory portion each have a duration of one-half of the whole period, results in a CO_2 increase which is about the same as when the breath is simply held for the whole period. And in the second place, the number of respirations does influence the percentage of CO_2 as seen by the percentage increase when two or more movements are made.

TABLE 3

The effect of varying the number of respiratory movements in unit time on the rise of CO_2 tension

10 seconds

HOLDING BREATH		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 1 MOVEMENT		INSPIRATION 2½ SECONDS EXPIRATION 2½ SECONDS 2 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.69–5.11	138	3.62–4.96	137	3.44–4.87	142
3.55–4.79	135	3.55–4.81	136	3.57–5.06	142
3.60–4.88	136	3.58–4.83	135	3.66–5.11	140
3.46–4.81	139	3.58–4.93	137	3.53–5.12	145
3.37–4.55	135	3.70–4.81	130	3.39–4.81	142
				3.42–4.83	141
Average = 137		Average = 135		Average = 142	

20 seconds

HOLDING BREATH		INSPIRATION 10 SECONDS EXPIRATION 10 SECONDS 1 MOVEMENT		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 2 MOVEMENTS		INSPIRATION 3⅓ SECONDS EXPIRATION 3⅓ SECONDS 3 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.84–5.80	151	3.77–5.47	145	3.52–5.60	159	3.55–5.94	167
3.62–5.67	157	3.81–5.65	148	3.49–5.71	163	3.56–6.04	169
3.52–5.48	156	3.71–5.79	156	3.61–5.87	163	3.60–6.08	169
3.51–5.51	157	3.51–5.37	153	3.66–5.86	160	3.67–6.06	165
		3.59–5.67	158	3.48–5.72	164		
		3.63–5.55	153				
		3.34–5.28	158				
Average = 155		Average = 153		Average ≐ 162		Average = 168	

30 seconds

HOLDING BREATH		INSPIRATION 7½ SECONDS EXPIRATION 7½ SECONDS 2 MOVEMENTS		INSPIRATION 3¾ SECONDS EXPIRATION 3¾ SECONDS 4 MOVEMENTS		INSPIRATION 3 SECONDS EXPIRATION 3 SECONDS 5 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.72–5.77	155	3.71–6.20	167	3.50–6.19	177	3.40–6.12	180
3.58–5.52	154	3.46–6.03	174	3.64–6.27	172	3.86–6.87	178
3.38–5.49	162	3.55–6.20	175	3.77–6.45	173	3.93–6.88	175
3.61–6.01	166	3.41–5.76	169	3.71–6.42	173		
3.40–5.44	160	3.72–5.90	156	3.32–6.04	182		
3.56–5.74	161	3.61–6.01	166	3.62–6.37	173		
Average = 160		Average = 168		Average = 175		Average = 177	

The explanation of the approximate same percentage of increase of CO_2 when the air is inspired for 5 or 10 seconds and then expired for an equal length of time, as when the breath is held for the whole time, is probably due to the longer duration of the negative pressure during the inspiration, which results in accelerating the rate of the CO_2 exchange. Probably also the blood stream is retarded while the breath is held and therefore less CO_2 is exchanged during the period although it is a longer time. It would seem that the CO_2 exchange takes place for the greater part during the actual inspiration of the air, that is, during the time that there is a negative intra-pulmonic pressure which, when the breath is quickly inspired and then held, is relatively short,—since the negative intra-pulmonic pressure quickly rises to atmospheric after inspiration—as compared with that existing when the actual act of inspiring the air lasts for 5 or 10 seconds.

The hindrance to the circulation during the period that the breath is held must play an important part, although Wardlaw does not admit that it does. Hill and Flack (6) also found a lower tension of CO_2 after holding the breath for a certain period than when the air was rebreathed. They explain this as being due to a mechanical obstruction of the circulation. Douglas and Haldane (7) also think that there is a slight circulatory block when the breathing is suspended. The findings of Ebert (8), contrary to the opinion of Wardlaw, do not seem to me to bear on the particular point of the retarded circulation during the time the breath is held. Ebert found that the flow of blood through the lungs was hastened during inspiration and retarded during expiration.

If the greater increase in carbon dioxide tension with the number of respiratory movements is due to the negative intra-pulmonic pressures during the actual act of inspiration as Wardlaw claims, then his results on varying the frequency of the respiratory movements and which he found not to affect the increase in the percentage of carbon dioxide are incomprehensible. For certainly the negative pressure exerted by the inspiratory portion of the three respiratory movements must be approximately three times as effective as that exerted by one respiratory movement. My results show that the frequency of respiratory movement does affect the velocity of the carbon dioxide exchange in the lungs so that, whatever its cause, it must act every time a respiratory movement is made.

The next series of experiments consisted in investigating the effect of varying the amplitude of the respiratory movements on the relative percentage of CO_2 in the rebreathed air. The percentage of CO_2 when the breath was held was compared with that found when it was freely rebreathed, and with that found when inspiratory and expiratory efforts were made with the glottis closed.

Wardlaw found by this method that the CO_2 of the alveolar air had the same tension when four respiratory efforts with the glottis closed were made in 20 seconds as when one or three normal respiratory movements were made. My results (see table 4) again differ in some particulars with his. In my experiments the air in the bag, after its CO_2 tension had been determined by analysis, was inhaled quickly and then, with the glottis closed, inspiratory and expiratory efforts were made, each for the stated number of seconds and for the stated number of times, and then the air quickly exhaled into the bag and its CO_2 percentage again determined.

Rebreathing the air, or performing inspiratory and expiratory movements with the glottis closed, results in about the same increase in CO_2—a little higher, probably due to the air being in the lungs and air passages all the time—over that obtained when the breath is held, as free rebreathing does (Cf. tables 3 and 4).

The number of respiratory movements in unit time thus influences the CO_2 tension when the glottis is closed as it does when it is open. The conclusion therefore seems obvious that the respiratory movements, or rather the inspiratory portions, hasten the rate of gaseous exchange.

When an act of inspiration or of expiration is made with the glottis closed the pressure in the lungs falls and rises with the rarefaction or compression of the contained air. Also at each inspiration there is, due to the increased negativity of intrathoracic pressure, an increase in the aspiratory action and an increase in the venous flow to the right heart, and consequently more blood thrown into the pulmonary circulation. In rebreathing into and out of a bag, the increase in the pulmonary circulation during inspiration must increase the velocity of the gaseous exchange. During expiration the decreased flow of blood and the consequent retarding of the velocity of the gaseous exchange do not play an important part in the comparative amount of CO_2 exchanged during rebreathing and when the breath is held, either when the air is kept in the lungs by closing the glottis during an expiratory movement or when the air is blown out into the bag. With the next inspiratory and expiratory movement the process is repeated. We would therefore expect when respiratory movements are made with the glottis closed, just as when the movements of free rebreathing are performed back and forth into a bag, that the percentage of CO_2 in the air would be greater than when the breath is simply held, because of the increasing effect upon the velocity of the gaseous exchange of the increased circulation or of the negative intra-pulmonic pressure occurring at each act of inspiration. The results in tables 3 and 4 show that this is indeed the case.

TABLE 4

The effect of varying the depth of the respiratory movements on the rise of CO_2 *tension*

10 seconds

HOLDING BREATH		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 1 MOVEMENT		INSPIRATION 2½ SECONDS EXPIRATION 2½ SECONDS 2 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.48–4.91	141	3.38–4.85	143	3.63–5.41	149
3.33–4.33	130	3.44–4.98	145	3.42–4.86	142
3.34–4.58	137	3.27–4.55	139	3.53–5.06	151
3.80–5.18	136			3.50–4.97	151
3.68–5.08	138			3.67–5.25	143
3.50–5.01	143				
Average = 138		Average = 142		Average = 146	

20 seconds

HOLDING BREATH		INSPIRATION 10 SECONDS EXPIRATION 10 SECONDS 1 MOVEMENT		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 2 MOVEMENTS		INSPIRATION 3⅓ SECONDS EXPIRATION 3⅓ SECONDS 3 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.78–5.86	155	3.66–5.78	158	3.43–5.66	165	3.32–5.68	171
3.27–5.00	153	3.40–5.20	153	3.66–5.82	159	3.47–5.79	167
3.56–5.33	149	3.76–6.14	165	3.67–6.13	167	3.37–5.70	169
3.56–5.52	155	3.54–5.35	154	3.54–5.49	155	3.25–5.59	172
3.62–5.29	146	3.41–5.35	157	3.47–5.79	167	3.33–5.73	172
3.65–5.55	152	3.68–5.59	152				
Average = 152		Average = 157		Average = 163		Average = 170	

Boothby and Peabody (9) in connection with the determination of the alveolar CO_2 by the Plesch-Higgins method found that the rate and depth of respiration while rebreathing do not play a very important rôle, in that the CO_2 tension after five moderately deep or five very deep respirations in 25 seconds was about the same as that obtained after 16 or 17 shallow respirations in the same time. The CO_2 tension, however, was considerably greater than when the breathing was "natural." It may be stated here that the CO_2 tensions which these authors give, (with the exception of the value obtained with five "natural" respirations in 25 seconds) are all quite high, and probably represent the CO_2 tension of the "venous pulmonary air" rather than that of the "arterial pulmonary air," as Henderson and Prince have suggested the determinations by the Plesch-Higgins method are likely to do.

Finally in order to ascertain whether variations in intra-pulmonic pressure have any effect, directly or indirectly, upon the rate of CO_2 exchange between the blood and the air in the lungs, a series of determinations of the CO_2 tension in the air was made after the breath had been held under positive and negative pressures. The results are shown in table 5. The CO_2 tension is the same after the breath has been held for 20 seconds under positive pressures of 10, 20 or 30 mm. Hg. as when it is held under atmospheric pressure. But when the breath is held under negative pressure the CO_2 tension shows a considerable increase.

These results are similar to those obtained by Wardlaw. Up to negative pressures of 10 mm. Hg. he found that the increase in CO_2 tension was proportional to the pressure. Above this pressure the increase in CO_2 was found to be practically constant for all pressures. Wardlaw obtained practically the same increase in the rate of the gaseous exchange when the breath was held under pressures greater than -10 mm. Hg. as when the air was rebreathed. This fact he cites as additional evidence that the increased respiratory exchange caused by the movements of breathing is not brought about by a quickening of the circulation. Now if the percentage of increase of CO_2 when the air is rebreathed three times in 20 seconds is noted (see tables 3 and 4), it is seen that it is only a little lower than when the breath is held under negative pressures for the same length of time (see table 5).

The bearing of these and Wardlaw's results of a similar kind on indicating that the increased gaseous exchange is not due to a quickening of the circulation, as Wardlaw claims, is not clear to me. The fact, which Wardlaw apparently neglects to consider, is not that the increased circulation necessarily increases the rate of gaseous exchange but that the decreased rate of circulation, or the diminution of the blood volume, when the breath is held, decreases the rate of gaseous exchange. During rebreathing the pressure conditions approach more nearly those holding under normal breathing where at each inspiration the circulation rate is hastened, and at each expiration, retarded.

It may be that when the negative intrapulmonic pressure is increased by holding the breath under a negative pressure, the retarding effect on the gaseous exchange due to the mechanical obstruction to the circulation is offset by the greater than normal negative intrapulmonic pressure.

TABLE 5

Comparison of the rise of CO_2 tension when the breath is held for 20 seconds under atmospheric pressure, and under various positive and negative pressures

ATMOSPHERIC		+ 10 MM. Hg		+ 20 MM. Hg		+ 30 MM. Hg	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.66-5.72	156	3.53-5.11	144	3.66-5.44	149	3.69-5.41	147
3.82-5.75	151	3.70-5.70	154	3.74-5.69	153	3.81-5.52	145
3.74-5.69	152	3.89-5.83	150	3.71-5.62	152	3.82-5.81	152
3.81-5.70	150	3.76-5.85	156	3.73-5.65	151	3.62-5.59	162
3.58-5.68	159	3.63-5.84	161	3.69-5.58	151	3.55-5.75	162
Average = 154		Average = 153		Average = 151		Average = 152	
ATMOSPHERIC		− 10 MM. Hg		− 20 MM. Hg		− 30 MM. Hg	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.71-5.75	155	3.70-6.17	167	3.75-6.24	167	3.51-6.02	172
3.72-5.74	155	3.55-6.12	172	3.75-6.21	166	3.46-6.19	176
3.36-5.24	156	3.69-6.39	173	3.43-6.21	181	3.56-6.35	178
3.59-5.56	155	3.77-6.43	171	3.85-6.40	171	3.33-5.99	180
3.78-5.71	151	3.61-6.04	167	3.66-6.42	175	3.73-6.51	175
Average = 154		Average = 170		Average = 172		Average = 176	

It might be thought that the higher tension of CO_2 after rebreathing air as compared with the tension attained after holding the breath was due to the influence of oxygenation. But, as Wardlaw's results show, the oxygen tension falls at a more rapid rate than that at which the CO_2 rises, and falls more rapidly when the air is rebreathed than when the breath is held. Furthermore when the breath is held long enough the CO_2 tension attains a certain fixed value while the oxygen tension continues to fall during the whole period for which the breath can be held or the air rebreathed.

Hill and Flack found too when the breath was held that the fall in the percentage of oxygen is greater than the rise of CO_2. Also that when oxygen is held instead of air, the tension of CO_2 at the breaking point is raised. Furthermore that when the air is rebreathed, the CO_2 goes higher and the oxygen tension lower than when the breath is held, and again that when oxygen is rebreathed a much higher tension of CO_2 is reached.

Now the higher tension of CO_2 reached when oxygen is held or rebreathed is undoubtedly due to the influence of oxygenation (see Christiansen, Douglas and Haldane), but it does not follow that the higher percentage of CO_2 attained after rebreathing than after holding the breath is due to the same cause, because the oxygen tension falls more rapidly in the former case and therefore the higher percentage of CO_2 could not be due to the forcing of CO_2 out by the higher tension of oxygen, but rather the de-oxygenation of the blood in the tissues would help the absorption of CO_2 and diminish its rise of pressure.

It seems to me that the results of Hill and Flack indicate clearly that holding the breath produces some mechanical obstruction to the circulation by the cessation of the respiratory pump. In rebreathing, the blood is circulated more freely and CO_2 is therefore more quickly, and in greater amount, removed from the tissues than when the breath is held.

DETERMINATION OF THE CO_2 TENSION OF THE VENOUS PULMONARY AIR

It is evident from the experiments described above, as well as from the work of Christiansen, Douglas and Haldane and that of Wardlaw, that neither holding the breath nor rebreathing air from and into a closed bag, yields CO_2 tensions which can be considered as representing the CO_2 tensions of the venous pulmonary air. They are therefore of no practical value except in so far as they can be compared with and controlled by other methods.

The next point to determine was whether the method of Henderson and Prince gives results which represent the CO_2 tension of the venous pulmonary air.

Their method contains two variations, viz., *a*, holding the breath for a certain number of seconds at each successive intermittent rebreathing, and *b*, inspiring it for 5 seconds and expiring it for 5 seconds every time that it is intermittently rebreathed. These two methods have been compared in some detail, examples of the results being shown in tables 6 and 7. In table 6 CO_2 tensions obtained after intermittent holding of the breath for 5, 10 and 15 seconds are given. The CO_2 in the air in my lungs seems to be in equilibrium with the blood when its tension reaches between 6.10 and 6.30 per cent, (average 6.24 per cent, or 44.9 mm. Hg.), which occurs after the 5th, 6th or 7th intermittent rebreathing. The longer the air is held at each intermittent rebreathing, the more rapidly does the CO_2 tension increase (see held for 5, 10 and 15 seconds and fig. 2), the final constant value is however the same.

TABLE 6

The effect of intermittent rebreathing—holding the breath—on the rise of CO_2 *tension*

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2					
	Held for 5 seconds	Held for 5 seconds	Held for 10 seconds	Held for 10 seconds	Held for 15 seconds	Held for 15 seconds
0	3.60	3.35	3.40	3.61	3.11	3.68
1	4.31	4.22	4.76	4.96	4.63	5.65
2	4.95	4.90	6.14	5.95	6.05	6.29
3	5.21	5.44	6.32	6.19	6.14	6.33
4	5.44	5.71	6.36	6.11	6.34	6.33
5	5.65	5.96	6.39	6.13	6.28	6.30
6	5.93	6.07	6.26			
7	6.11	6.09	6.26			
8	6.13	6.11	6.21			
9	6.10	6.10				
10	6.12					

TABLE 7

The effect of intermittent rebreathing—inspiring for 5 seconds, and expiring for 5 seconds—on the rise of CO_2 *tension*

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2				
	1 respiration	1 respiration	2 respirations	2 respirations	3 respirations
0	3.41	3.57	3.31	3.19	3.61
1	4.77	4.92	5.06	5.08	6.35
2	6.01	5.90	6.10	6.03	6.79
3	6.13	5.94	6.47	6.50	6.91
4	6.20	6.11	6.78	6.58	7.12
5	6.33	6.09	6.63	6.56	7.12
6	6.31	6.08	6.64	6.63	7.10
7	6.28	6.10	6.70		
8			6.61		
9			6.63		

Every time that the air in the bag was rebreathed it must have been diluted to a small extent by the air in the dead space and to a slighter extent by the residual air, at least after the percentage of CO_2 in the rebreathed air had become greater than that of the arterial pulmonary air. Determination of the CO_2 tensions of the alveolar air, that is the last portions of an expiration after the air had been intermittently rebreathed until the CO_2 tension had become constant, by the method of Henderson and Morriss (10) gave, however, practically the same values for the CO_2 tension as analysis of the "mixed air" did.

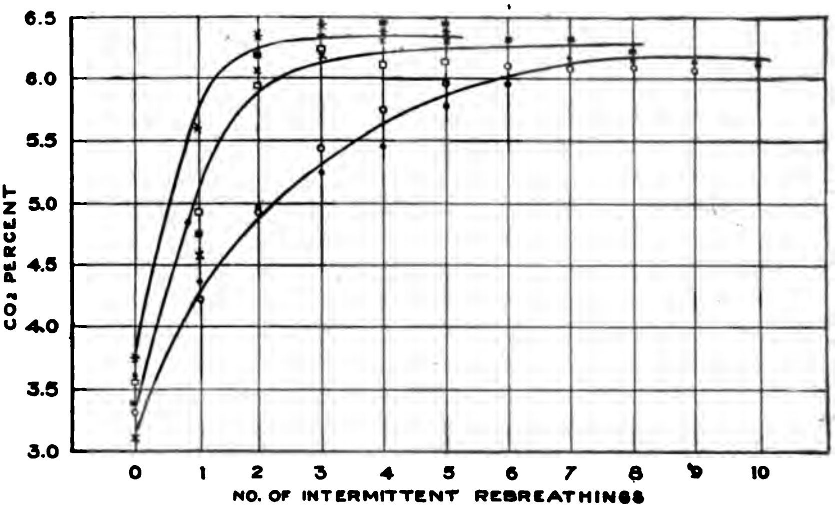

Fig. 2. The effect of intermittent rebreathing—holding the breath—on the rise of CO_2 tension; $\overset{\bullet}{\circ}$ when the breath is held for 5 seconds, $\overset{\blacksquare}{\square}$ when the breath is held for 10 seconds, $\overset{\times}{\bigstar}$ when the breath is held for 15 seconds.

Christiansen, Douglas and Haldane point out the marked influence that oxygenation has upon the dissociation curve of CO_2. In the lungs the amount of CO_2 given off is increased by about 50 per cent, while in the tissues the oxygen reduces by about 40 per cent the amount of CO_2 given off to the venous blood. The difference therefore between the CO_2 tensions of arterial and venous blood is reduced by about 40 per cent, and these authors had to take this into consideration in the determination of the venous CO_2 by their method. Boothby and Sandiford (3) also took care that there was sufficient oxygen in the mixtures which they held in the lungs to insure complete saturation of the Hb. In the method proposed by Henderson and Prince for the determination of the venous CO_2 tension the influence of oxygenation does not come into consideration.

The CO_2 tension of my arterial pulmonary air is 5.53 per cent. There is therefore a difference of 0.71 per cent (6.24–5.53), or 5.1 mm. Hg. (44.9–39.8), between the CO_2 tension of my arterial and of my venous blood, a figure quite close to the average differences found by Christiansen, Douglas and Haldane.

As mentioned above, Henderson and Prince suggested, as a variation on holding the breath every time that it was intermittently rebreathed, that it be inspired for 5 seconds and expired for 5 seconds "one or more times." This variation has been repeated to see whether it would give the same values for the CO_2 tensions that holding the breath for 10 seconds does. When, instead of holding the lungs full for 10 seconds, the air from the bag is inspired for 5 seconds and expired for 5 seconds, once, the same value for the CO_2 tension is found, (Cf. table 6, "held for 10 seconds" and table 7 "1 respiratory movement;" also see table 3). Holding the breath, after a sharp inspiration from the bag, for 5 seconds results in a percentage increase of CO_2 less than that resulting from holding the breath for 10 seconds, but inspiring continually for 5 seconds followed by an expiration of the same length of time results in a percentage increase which is greater than that obtained when the breath is held for 5 seconds and practically the same as when it is held for 10 seconds.

But when the number of times that the air is rebreathed in this way, by inspiring for 5 seconds and expiring for 5 seconds, every time that the air is taken into the lungs from the bag, is more than one, it is found that the rate of CO_2 increase is greater, as well as the final constant value attained (see table 7 and fig. 3). This is, of course, just what might have been expected from the results that have been described above on rebreathing as compared with holding the breath, as well as from the fact that the total length of time that the air is rebreathed is considerably increased. It is interesting to note that a constant CO_2 tension, higher as it is, is however reached.

The statement by Henderson and Prince regarding this variation is ambiguous. They probably did not mean that the air should be inspired and expired in this way more than once at each successive intermittent rebreathing.

The application of the CO_2 tension of the venous pulmonary air, as well as that of the arterial pulmonary air, to the Plesch-Higgins method is of clinical importance. Knowing the venous CO_2 tension, this application can be made with a degree of certainty. As has been shown above the length of time that the breath is held in the lungs, as well as the number of respiratory movements in unit time, have an influence on the CO_2 tension of the expired air.

Reference was made above to the results of Boothby and Peabody (9) on the relation of the CO_2 tension to the rate and depth of respira-

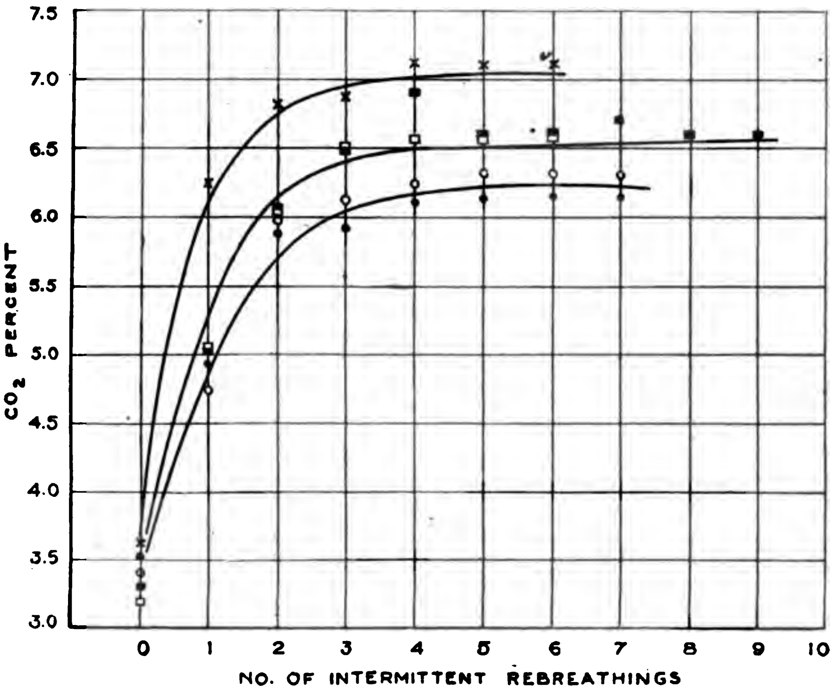

Fig. 3. The effect of intermittent rebreathing—inspiring for 5 seconds and expiring for 5 seconds—on the rise of CO_2 tension; ● ○ when one respiratory movement is made, ■ □ when two respiratory movements are made, × when three respiratory movements are made.

tion, and the suggestion made that the values of the CO_2 tensions which they obtained by the Plesch-Higgins method approximate the venous rather than the arterial, as compared with the tensions obtained by the Haldane method. Also reasons for doubting the validity of Wardlaw's results concerning the frequency of respiratory movement have also been given above, as well as evidence showing that increasing the number of respirations in a certain period of time—keeping the amplitude as constant as possible—raises the CO_2 tension of the expired air. Henderson and Prince (1) expressed the opinion that the Plesch-Higgins method of determining the alveolar CO_2 gives results which are too high. Further, that by this method of rebreathing the portion of the curve of CO_2 percentage where it begins to become level represents the CO_2 tension of the tissues. Table 2 and figure 1 of the present paper show that the beginning of the nearly horizontal part of the curve, or the approximate constancy of CO_2 comes after the 9th rebreathing, with an average CO_2 tension of 7.15 per cent. The CO_2 tension of my arterial pulmonary air is about 5.53 per cent. This tension is reached by the method of continuous rebreathing, somewhere between the 2nd and 3rd rebreathing (see table 2, 3rd and 4th, if the air expired into the bag be considered as having already been rebreathed once), and the venous CO_2 tension (6.24 per cent) is usually passed between the 4th and 5th rebreathing (or 5th and 6th for the above stated reason).

One of the regular exercises of the class in physiology in this school consists in the determination of the gas tensions of the arterial pulmonary air by the Haldane-Priestley, Henderson and Morriss and the Plesch-Higgins methods. The results obtained by Mr. B. E. Read, one of the members of the present year's class, are as follows. His arterial CO_2 tension by the Haldane-Priestley method is 5.72 per cent, by the Henderson and Morriss, 5.77. The CO_2 tensions after continuous rebreathing once every 5 seconds are given in table 8. The values given are the average of four or more determinations on separate rebreathings. The air rebreathed was expired into a bag, and was always somewhat more than the tidal volume in amount. After the air has been rebreathed twice, the CO_2 tension reaches that determined by the Haldane and Henderson methods. (If the air expired into the bag be considered as already having been rebreathed once, then the arterial CO_2 tension is reached after the 3rd continuous rebreathing).

In addition B. E. R. has determined the CO_2 tension of his venous pulmonary air by the method of intermittent rebreathing. This was found to be about 6.34 per cent. As seen from the values for continuous rebreathing given in table 8, the venous CO_2 is reached after the 3rd continuous rebreathing, (or the 4th, for the above given reason).

The following method of obtaining the CO_2 tension of the venous pulmonary air and which is applicable to clinical patients, is suggested. A bag sufficiently large to hold enough air to fill the lungs when inhaled is filled with fresh air, by water displacement or by a pump. This air is taken into the lungs after they have been emptied as far as possible, and then immediately exhaled into the bag and a sample analyzed for CO_2, (found, in my case, to vary between 3.25 and 4.0 per cent). Or the subject can exhale lightly, with pauses, two or three times into the bag, inspiring from the outside air, and the air in the bag then be analyzed for CO_2. This air should now be intermittently rebreathed,—with sufficiently long pauses between successive rebreathings to allow the circulation and respiration to become normal,—according to the Henderson and Prince method, of inspiring for 5 seconds and expiring for 5 seconds, until on subsequent rebreathings and analyses a constant CO_2 percentage is reached. Since the same results have been shown to be obtained when the breath is held for 10 seconds as when the air is inspired for 5 seconds and expired for 5 seconds once for each intermittent rebreathing (see tables 6 and 7), the latter method, owing to the greater ease with which it can be carried out by sick patients is preferable for them.

TABLE 8

The rise of CO_2 tension after continuous and intermittent rebreathing

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2		
	B. E. R.		E. F. A.
	Continuous rebreathing	Intermittent rebreathing	Intermittent rebreathing
1	4.62	4.41	4.34
2	5.73	5.27	5.69
3	6.21	5.93	6.39
4	6.82	6.31	6.43
5	6.91	6.34	6.60
6			6.56

Furthermore by this same procedure the CO_2 tension of the arterial pulmonary air can be approximately determined. As tables 6 and 7 show, when the air after inspiring it from the bag, is held for 10 seconds and then expired into the bag; or when it is inspired for 5 seconds and expired for 5 seconds, at each intermittent rebreathing, a CO_2 tension approximately equal to that of the arterial pulmonary air is passed between the 1st and 2nd intermittent rebreathing. By adding the tensions found after the 1st and 2nd rebreathing, and halving them, the approximate CO_2 tension of the arterial pulmonary air can be obtained. The average obtained when the breath is held for 10 seconds is 5.46 per cent; when an inspiration and expiration, each lasting 5 seconds, is made at each intermittent rebreathing, an average of 5.41.

An even more approximate determination of the CO_2 tension of the arterial pulmonary air can be obtained by taking the average of the CO_2 tensions of the first two rebreathings, and the average of the first three, and then taking the average of these. When the breath is held for 10 seconds at each intermittent rebreathing (see table 6) a value of 5.59 is thus obtained, and when the air is inspired for 5 seconds and expired for 5 seconds once at each intermittent rebreathing (see table 7) a value of 5.54 per cent. By applying this calculation to the results obtained on two other subjects (see table 8) a percentage value of 5.72 is obtained for B. E. R., which is identical with the CO_2 tension obtained by the Haldane method; and of 5.24 for E. F. A., whose arterial pulmonary air by the Haldane method has a percentage value of 5.36.

SUMMARY

1. The rise of CO_2 tension in the lungs when the breath is held is compared with that when the air is rebreathed.
2. The CO_2 in both increases at a gradually decreasing rate until it reaches an apparent maximum. The rate of increase and the final value attained are higher when the air is rebreathed than when it is simply held in the lungs. The time that the air can be rebreathed is also longer than that for which it can be held, although the CO_2 is higher.
3. The method suggested by Henderson and Prince for the determination of the venous CO_2 tension has been carefully gone over and the two variations suggested by them of intermittent rebreathing compared.
4. The Plesch-Higgins method of determining the CO_2 tension of the alveolar air is shown to give results which are too high when the air is rebreathed more than two to three times, and approximately equal to the venous CO_2 tensions when four to five rebreathings are made.
5. A method for determining the venous CO_2 tension (slightly modified from that of Henderson and Prince) and applicable to clinical patients, is suggested, as well as for the calculation of the approximate arterial CO_2 tension.

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LOCATION OF THE ADRENALIN VASODILATOR MECHANISMS

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It has been demonstrated (1) that adrenalin vasodilatation in the limb can be produced by stimulation of other than peripheral structures. It has also been shown that intestinal vasodilatation from adrenalin involves either the collateral ganglia or the central nervous system. These facts were established, *a*, by cutting the nerves to the limb in the one case and by destroying the ganglia in the other; *b*, by perfusion experiments in which the organ is cut off from the body circulation and the nerves left intact, adrenalin being injected into the jugular vein.

Previous to the present research we had found evidence which indicated a difference between the type of mechanism causing vasodilatation in the limb, as an example of skeletal muscle, and that producing like effect in the intestine: viz., 1, small doses of adrenalin produce constriction in the intestine and dilatation in the limb while larger doses produce the reverse effect, i.e., dilatation in the intestine and constriction in the limb (1, p. 366); 2, greatly increasing the dose above that causing intestinal dilatation does not produce predominant constriction in the intestine; 3, the intestinal vasodilator mechanism develops later than the corresponding mechanism for the limb (2).

In the present research we have attempted to determine the location of these mechanisms.

The procedure followed was to destroy different portions of the brain and spinal cord, to remove the sympathetic ganglia or to destroy the dorsal root ganglia of the nerves from the organ investigated and then to ascertain the activity of the vasodilator mechanisms according to methods described in previous investigations (1).

Removal of cerebrum and cerebellum. A cat (2.8 kgm.) responded to 0.2 cc., 1:100,000 adrenalin with a fall in blood pressure of 19.3 per cent (150 mm. to 121 mm.). Nine minutes after removal of the cerebrum a similar dose of adrenalin produced the same percentage drop in blood pressure (114 mm. to 92 mm.) This indicates that the adrenalin vasodilator mechanisms are not in the cerebrum, at least those which control the vessels of skeletal muscle and are called into play by small doses of adrenalin. In order to confirm our conclusion in regard to the position of these, we studied the volume changes in the hind limb of one cat and one dog. Dilatation of the limb of the cat from adrenalin occurred after decerebration as well as before. A similar result was obtained in the dog after removal of both cerebrum and cerebellum.

Blood pressure changes, however, do not indicate the action of the intestine, therefore in order to discover whether the intestinal mechanism was present in the cerebrum the volumetric method was necessary. In both animals the intestinal dilatation thresholds were determined before decerebration. The cerebrum was destroyed in the cat, then the same dose of adrenalin was injected as before with like result, showing that the intestinal mechanism was not in the cerebrum. The dog had both the cerebrum and the cerebellum removed without interfering with the intestinal dilatation.

We are justified, therefore, in concluding that neither type of the adrenalin vasodilator mechanisms is present in the cerebrum or cerebellum.

Destruction of the medulla. Our next step was to destroy the medulla. This was done in those animals which had served for the cerebral experiments, and in some others in which the brain was pithed in one operation. (In either case the ether was immediately discontinued.) Destruction of the medulla always produced a reversal in the blood pressure response to adrenalin, in none was there a fall in blood pressure (four dogs and eight cats). It appeared, therefore, that the dilator mechanisms might be situated in this region of the central nervous system. A typical example is as follows:—before pithing the brain, 0.2 cc., 1: 100,000 adrenalin produced a 14 per cent fall in blood pressure in a cat (166 mm. to 152 mm.); twice the amount produced a 30 per cent fall. After pithing, the same doses of adrenalin produced 10 per cent and 32 per cent rises in blood pressure, respectively (from 78 mm.). One of us has shown (3), however, that a decrease in the blood pressure (e.g., by hemorrhage) is enough in itself to produce a similar result. The reversal in the reaction in this case then was not necessarily due to destruction of the dilator mechanisms. We sought an answer to this question by a study of the volume changes of the organs. The dilatation of the limb muscles, recorded with the plethysmograph, in two dogs and four cats followed the curve of the rise in blood pressure and seemed to be passive. Intestinal volume changes were recorded in seven subjects (two dogs and five cats). In all but one the dilatation was active, independent of the rise in pressure.

In order to exclude all possibility of passive dilatations, the hind limb of a cat was perfused with warm oxygenated Ringer's solution through the common iliac artery. The abdominal aorta was clamped high up to prevent anastomoses. The vena cava was tied and an outlet made in the common iliac vein (1, p. 360). The response of the perfused limb to various doses of adrenalin injected into the general circulation was noted, after which the brain was pithed and the injections repeated. The resulting dilatation was in every case as marked as before. Similarly the perfused hind limb of a dog responded by dilatation as well after destruction of the brain as before. A perfused intestinal loop of a brainless dog dilated when adrenalin was injected into the general circulation.

Cervical cord. Having failed to locate the vasodilator mechanisms in the medulla or higher, their presence in the cord of the cervical region seemed very doubtful. The upper part of the central nervous system down to the thoracic cord was destroyed by pithing in a dog and a cat. The limb mechanism in the dog was active, as determined by the plethysmograph after pithing. The intestinal reaction of the dog was not studied but in the cat it was still present. It must be concluded that both mechanisms are below the cervical cord.

Thoracic cord. After destruction of the central nervous system as far down as the mid-thoracic region the adrenalin vasodilator mechanism for the intestine still worked in every case (four cats and one dog). It appeared, therefore, that the intestinal mechanism must be located below this region.

The cord was next pithed to the lumbar region in three cats and one dog. Adrenalin still caused intestinal dilatation in all cases, though in the dog the dilatation was not as marked as before. Therefore the mechanism for adrenalin vasodilatation in the intestine appeared to lie outside of the brain and spinal cord (fig. 1).

The adrenalin vasodilator mechanism for the hind limb is not located in the thoracic cord. This was proved in both normal and perfused limbs by destruction of the brain and cord. In the animals with the normal limb (two cats and one dog) one seemed to respond by passive dilatation while the others were active. To avoid passive effects the hind limb was perfused after destruction of the brain and cord to the lumbar level, (one cat and one dog). Injection of adrenalin into the jugular vein caused dilatation in the perfused limb in each case.

Lumbar cord. The brain and spinal cord were completely pithed in two cats. Adrenalin produced dilatation in the hind limb in both. However, this seemed to be passive. The lumbar and sacral cord only were destroyed in a third cat without preventing dilatation of the hind limb from adrenalin. Where the limb effects appear to be passive a distinction can be shown by using a denervated limb. The latter gives earlier and more marked constriction than does the normal limb (fig. 1). We again found it necessary to resort to perfusion experiments. In addition the pithing was done with a stiff brush to insure complete destruction of the cord.

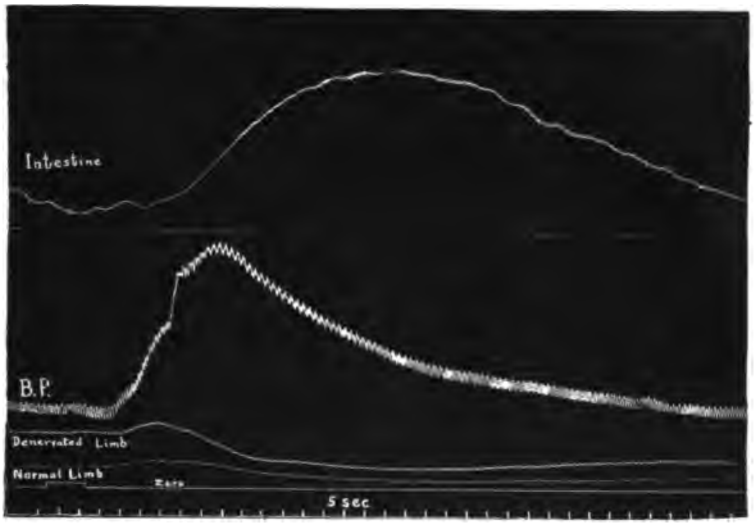

Fig. 1. The reaction of normal limb, denervated limb and intestine in a cat (weight 3 kgm.) to 3 cc., 1: 100,000 adrenalin after destruction of the brain and spinal cord. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

The lumbar and sacral regions of the cord were destroyed in two dogs. One hind limb was perfused and the abdominal aorta clamped. In both experiments good dilatations were obtained from injecting adrenalin into the jugular vein.

The sacral, lumbar and lower half of the thoracic cord were destroyed in two cats. The perfused hind limb in each case dilated when adrenalin was injected into the jugular vein (fig. 2). The dose of adrenalin necessary to do this was larger than is the case in a normal limb, perhaps because of the faulty circulation in the lumbar region caused by clamping the aorta above the bifurcation. We do not find the same difference after the operation in dogs as in cats, owing no doubt partly to the great number of anastomoses in a larger animal.

These results were confirmed by experiments in which the connection between the central nervous system and the limb under observation was severed, after which no reduction was found in the dilatation caused by adrenalin. We chose dogs for this operation. The spinal nerve roots were exposed on one side by removing the laminae and part of the transverse processes, but not the spines. Bleeding from the sinuses was stopped by hot saline packs from time to time. In the first experiment both dorsal and ventral roots of the sacral and lumbar regions were cut close to the cord on one side. The limb of that side was placed in a plethysmograph and perfused. The aorta and vena cava were tied. Injection of 0.5 cc., 1: 20,000 adrenalin into the jugular vein caused a pronounced dilatation of the perfused limb, in spite of the low blood pressure which had resulted from hemorrhage, and succeeding doses of the same strength had a similar effect. 2 cc., 1: 20,000 adrenalin caused dilatation which persisted for some time

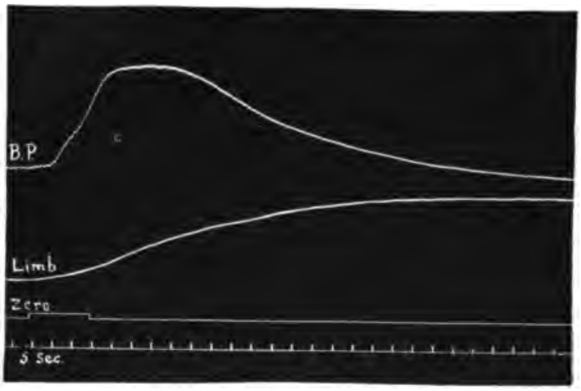

Fig. 2. Dilatation of a perfused hind limb in a cat (weight 2.5 kgm.) to 1 cc., 1: 10,000 adrenalin injected into the jugular vein after complete destruction of the spinal cord downward from the eighth thoracic level. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

(fig. 3). A second dog was studied in a similar manner with the same result.

We therefore came to the conclusion that the vasodilator mechanism for the hind limb could not be situated within the central nervous system but must lie either in the sympathetic or in the dorsal root ganglia or, perhaps, in both.

Location of the mechanism for skeletal muscle. Study of the sympathetic ganglia was next made. The right hind limb of a dog (26.0 kgm.) was placed in a plethysmograph. The last five lumbar and the first sacral sympathetic ganglia were destroyed on the right side. The limb was next completely shut off from the circulation and perfused with warm oxygenated Ringer's solution. Injection of 1.5 cc. of 1:20,000 adrenalin into the jugular vein caused slight dilatation of the perfused limb, while after twice the dose the dilatation was marked. A second animal was studied after destruction of the last five lumbar and the first two sacral sympathetic ganglia. The dilatation from

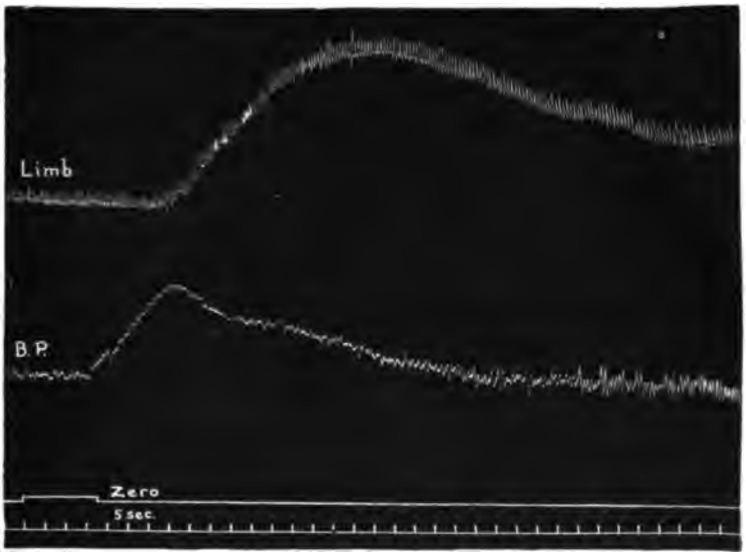

Fig. 3. Reaction of the perfused hind limb of a dog (weight 12.6 kgm.) to 2 cc., 1:20,000 adrenalin injected into the jugular vein after cutting both dorsal and ventral roots central to the dorsal root ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

adrenalin was very great (fig. 4). In a third dog both sympathetic chains were completely destroyed on both sides from the third lumbar ganglion downward. Adrenalin, injected into the general circulation, caused the perfused limb to dilate as in the other experiments. The animals were always examined at the completion of the experiments to ascertain the limit of gangliar destruction.

These experiments made it appear that the limb mechanism was not in the sympathetic ganglia but in those of the dorsal roots. To determine this we approached the question in another way. The dorsal and ventral roots of all lumbar and sacral nerves were cut central to the dorsal root ganglia on the right side. The right hind limb, after being placed in a plethysmograph, was completely cut off from the general circulation and immediately perfused. Adrenalin injected into the jugular vein caused dilatation of the perfused limb. The next step was removal of all dorsal root ganglia supplying the perfused limb. Following this operation injection of adrenalin into the jugular vein produced an effect on the perfused limb similar to that occurring before removal of the ganglia (see fig. 5). This was repeated in three dogs with the same result each time except that in one animal, in which

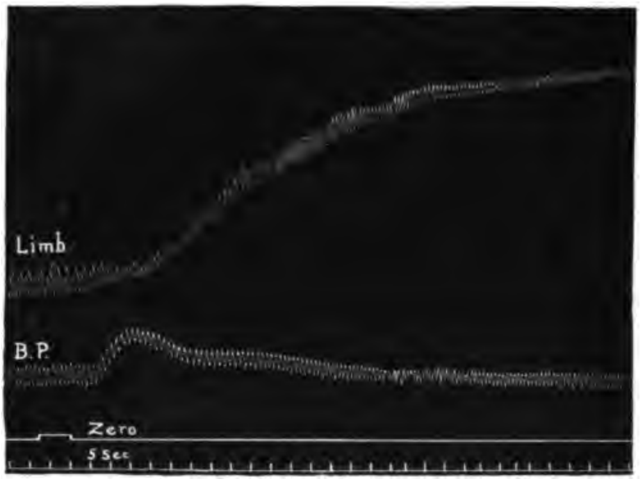

Fig. 4. Dilatation in the perfused hind limb of a dog (weight 21.6 kgm.) from the injection of 4 cc., 1: 20,000 adrenalin into the jugular vein, after removing the last five lumbar and the first two sacral sympathetic ganglia on the same side. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

the blood pressure became quite low after removal of the dorsal root ganglia, somewhat larger doses of adrenalin were required to produce dilatation as large as before; this may perhaps be due to the poor circulation to the sympathetic ganglia because of the clamp on the abdominal aorta and the general low blood pressure. We have indeed evidence of interference with the circulation of blood to the sympathetic ganglia in the delayed dilatation of the limb, i.e., in some animals the limb began to dilate long after the beginning of the change in blood pressure.

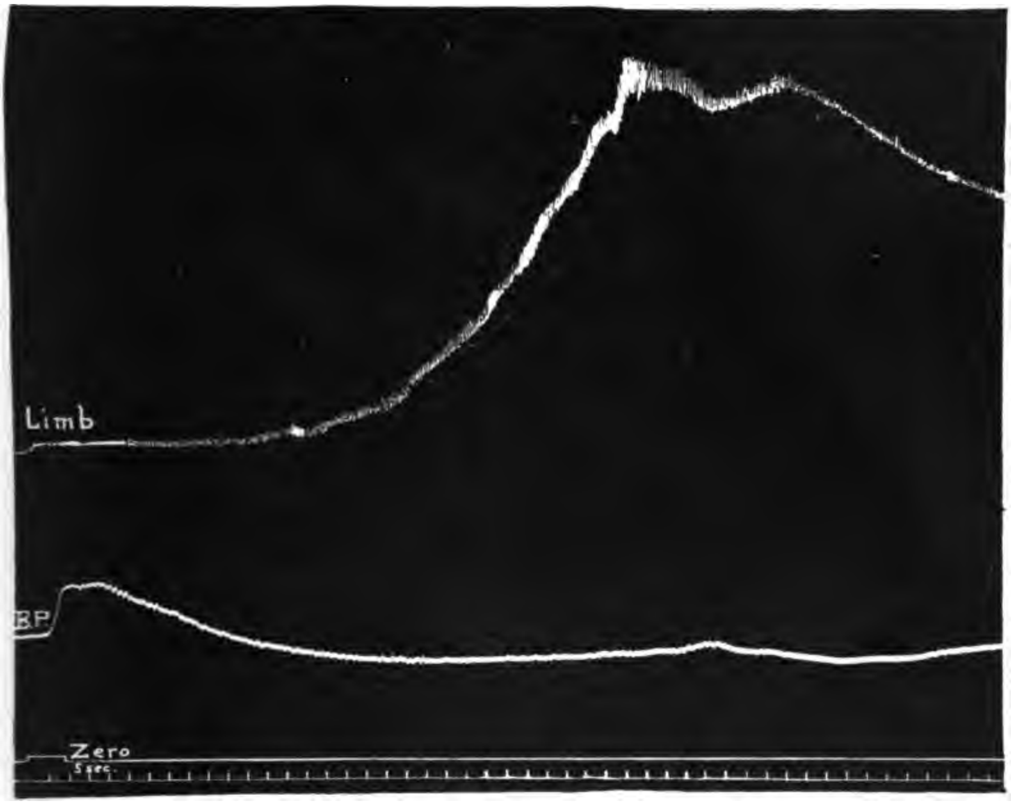

Fig. 5. Dilatation of a perfused hind limb (dog 9.0 kgm.) due to the injection of 2 cc., 1:20,000 adrenalin into the general circulation. All dorsal root ganglia had been removed after cutting the dorsal and ventral nerve roots in the whole lumbar and sacral region on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

Having shown that the adrenalin vasodilator mechanism for the hind limb is in part at least to be found in the sympathetic ganglia, we next proved that the dorsal root ganglia were also in part responsible for the dilatation when adrenalin was injected. In the experiments where the sympathetic chains to the perfused limb had been completely destroyed, the remaining adrenalin vasodilator mechanisms must have been either in the dorsal root ganglia or in the spinal cord. Since from the results of our experiments on destruction of the central nervous system we were convinced that it did not contain the seat of the reaction, we wished to have definite proof that this was to be found in the dorsal root ganglia. This proof we got by destroying both abdominal and sacral sympathetic chains and cutting both dorsal and ventral roots central to the dorsal root ganglia in the whole lumbar and sacral region on the side from which the perfused limb received its supply.[1] Four dogs were studied after the above operation. In each case adrenalin injected into the general circulation caused dilatation of the perfused limb (see fig. 6). In two of the animals we then removed the dorsal root ganglia, whereupon adrenalin when injected into the general circulation failed to produce any effect upon the perfused limb.

We were able to confirm the location of the adrenalin vasodilator mechanisms for the hind limb in both sympathetic and dorsal root ganglia by the direct application of adrenalin to them.

The influence of adrenalin upon the sympathetic ganglia of the lumbar region was studied in three cats. The last two lumbar ganglia were exposed by careful dissection. A small funnel was clamped in such a position that the outlet was over one of the ganglia so that small amounts of adrenalin, poured down the funnel, bathed it. Between applications the adrenalin was washed out with isotonic salt solution and taken up with absorbent cotton. The volume of the limb was recorded by means of a plethysmograph. In the first animal a 1: 100,000 solution of adrenalin produced a good dilatation of the limb with hardly any blood pressure change. A 1:10,000 solution produced marked constriction of the limb together with a steady rise in blood pressure. We consider that this constriction was not necessarily a local effect at the ganglion because of the pronounced blood pressure change which accompanied it. The second animal gave dilatation of the limb upon the first application of 1: 100,000 adrenalin, but later applications of the same concentration were without effect.

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[1] We were unable to obtain satisfactory results in cats in most cases after exposure of the dorsal root ganglia, as the following experiments show. We cut dorsal nerve roots central to the ganglia in two cats on one side. In one all roots were cut from the sacral to the mid-thoracic, in the other all to the thoracic level were cut. Then the hind limb on the corresponding side was placed in a plethysmograph and perfused. In neither animal could dilatation of the perfused limb be obtained from injection of adrenalin into the general circulation. In a third cat the lumbar and sacral cord was merely exposed, after which a hind limb was placed in a plethysmograph and perfused. Even in this case no dilatation could be obtained in the perfused limb when adrenalin was injected into the general circulation, although the blood pressure was about normal (142 mm.) In the two preceding cases the blood pressure was so low that it was thought possibly a factor, (18 mm.)

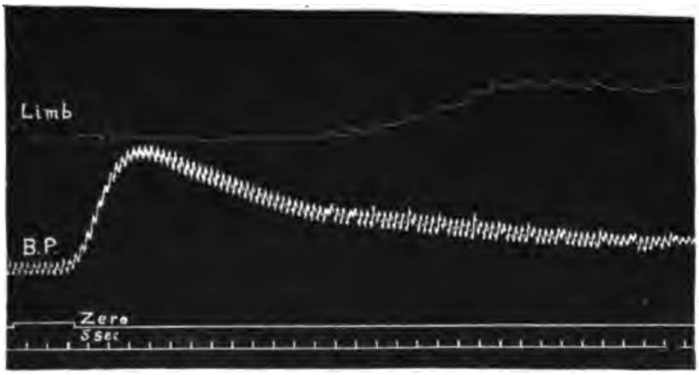

Fig. 6. Dilatation of a perfused hind limb (dog 14. kgm.) from the injection of 5.5 cc., 1: 10,000 adrenalin into the jugular vein. All sympathetic ganglia on both sides in the lumbar and sacral regions had been destroyed and all dorsal and ventral nerve roots central to the dorsal root ganglia had been cut below the thoracic level on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

In the third animal a 1: 100,000 solution had no effect. A 1: 10,000 solution caused a slight dilatation of the limb and a fall in blood pressure from 105 mm. to 102 mm. A 1: 5000 solution caused a more pronounced diltation of the limb and a fall in blood pressure of 25 mm. (fig. 7). A 1: 1000 solution caused marked dilatation of the limb followed later by constriction. The blood pressure change in this case was a pure fall of 21 mm.

It was more difficult to produce dilatation of the hind limb by the application of adrenalin to the dorsal root ganglia. Three dogs were studied. The lower lumbar ganglia were used, the sheaths covering them were slit and sometimes the ganglia themselves in order to permit better access of the adrenalin. In all cases both dorsal and ventral roots were cut central to the ganglia to prevent any possible effect from the cord. The solution of adrenalin was washed away with isotonic salt solution between each application. In the first experiment, a solution of 1: 10,000 adrenalin produced a doubtful dilatation. A second dose of the same concentration produced no effect nor did stronger concentration cause any change. In a second experiment we met with greater success. Although 1: 10,000 solutions produced no effect, those of 1: 1000 caused dilatation in the limb and repeated applications of solutions of this concentration always produced the same effect. In a third case dilatation of the hind limb was obtained time after time upon application of 1: 1000 adrenalin to the lower dorsal root ganglia (see fig. 8).

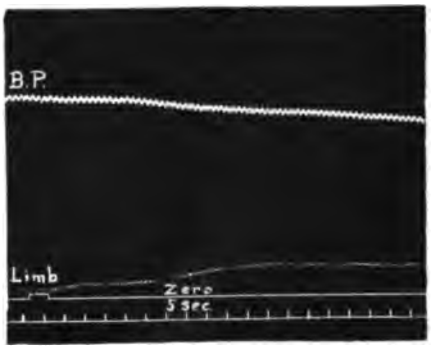

Fig. 7. Dilatation of a hind limb due to direct application of 1: 5000 adrenalin to the sixth and seventh lumbar sympathetic ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

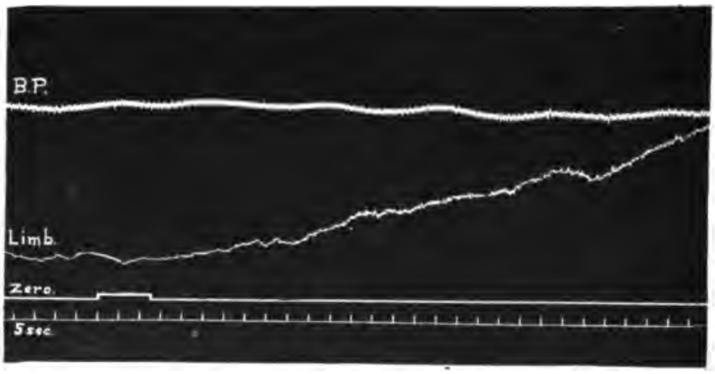

Fig. 8. Dilatation of a hind limb due to direct application of 1: 1000 adrenalin to one of the lower lumbar dorsal root ganglia. Dog, 16 kgm. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

Location of the intestinal mechanism. We have previously shown (4) that the intestinal vasodilator mechanism does not function after destruction of the semilunar and superior mesenteric ganglia. At that time we stated that cutting of the splanchnic nerves produced the same result, judging from the result of the one experiment of this kind (the other experiments of the series were gangliar destruction).

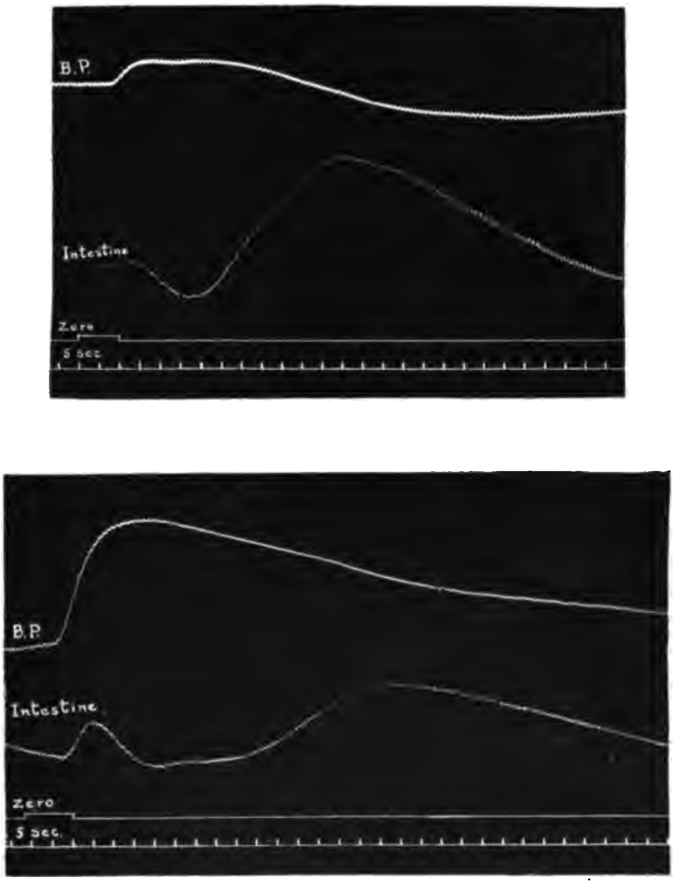

Fig. 9. Dilatation of intestine from adrenalin persists after cutting all splanchnic fibers, though it may be reduced. Upper record is the response to 0.5 cc., 1: 10,000 adrenalin before cutting the splanchnic fibers. Lower record is the response to 1 cc., 1: 10,000 adrenalin after cutting the splanchnic fibers in the same animal. (Cat weight 2.5 kgm.) Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

It now appears from further experiments that cutting the splanchnics does not necessarily do away with the dilatation. We divided the splanchnic nerves in five cats and took records of the reaction of the intestine to adrenalin. In one the reaction was constriction only; the remaining four gave dilatation as before except that in one animal it was less marked (fig. 9) Destruction of the semilunar and superior mesenteric ganglia in one of these greatly reduced the dilatation from adrenalin but did not quite abolish it. Since, however, section of all nerves in the stalk of this loop did not prevent a small amount of dilatation, we concluded that some small part of the previous dilatation had been either passive or else due to stimulation of peripheral structures.

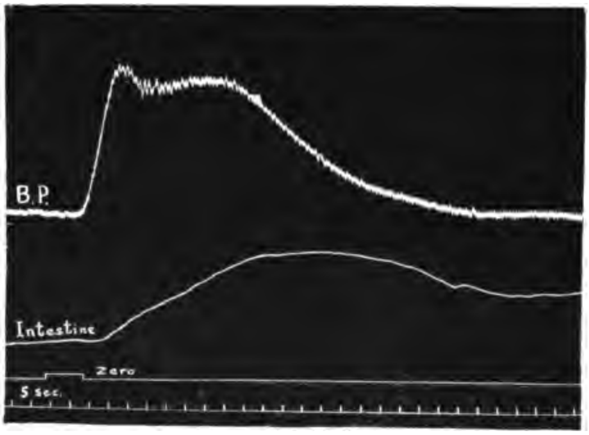

Fig. 10. Dilatation of a perfused loop of intestine of a dog (weight 13.5 kgm.) caused by the injection of 2 cc., 1: 10,000 adrenalin into the jugular vein. Postganglionic fibers intact but all central nervous connection destroyed by cutting the splanchnic fibers. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

In order to eliminate peripheral effects we perfused loops of intestine by the method already described (1, p. 360). All splanchnic nerve fibers and in some cases the vagi were cut. Six dogs were studied by this method. In every animal adrenalin injected into the jugular vein caused dilatation of the perfused loop of intestine, as great in amount as that usually obtained in perfused loops of which the central nervous connection was intact (fig. 10). In two dogs the dilatation was often preceded by constriction. Cutting the nerves in the stalk of the perfused loop did away with all effects of the injection. Isolation of the collateral ganglia from the central nervous system was verified in each instance by post-mortem dissection. Two cases showed incomplete section of the lesser splanchnics. In the remaining four the destruction of central nervous connection with the ganglia was found to be complete.

If sympathetic ganglia control the adrenalin vasodilatation in the intestine, it is natural to suppose that suitable doses of nicotine should reduce the dilatation by paralyzing the sympathetic nerve cells. This was found to be the case. A cat (3.2 kgm.) was given intravenously a total of 2.1 cc. of a 0.1 per cent nicotine solution divided into four doses. The dilatation in the intestine from adrenalin was smaller in amount after nicotine than before. A second cat (2.6 kgm.) gave a similar result after an intravenous dose of nicotine (1.7 cc. of a 0.1 per cent solution). The intravenous injection in a third animal prevented the intestinal dilatation altogether (fig. 11). In a fourth cat dilatation was prevented by painting the superior mesenteric ganglion with a 1 per cent nicotine solution.

Vasodilatation of the intestine, therefore, is apparently caused by the action of the adrenalin upon some structure in the superior mesenteric ganglion. As an added proof of this we have been able to cause dilatation of the intestine by the direct application of adrenalin solution to the superior mesenteric ganglion. The intestine of a cat was placed in an oncometer. The mesentery was cut and separated from the superior mesenteric ganglion in such a way that a pocket could be made by engaging the cut surface of the mesentery with haemostats, to form a pool of the solution of adrenalin around the ganglion. The solution was simply poured into the pocket and between each application it was washed away with normal saline solution, which was afterwards removed by sponging. The following results were obtained: A small dilatation of the intestine was produced by a 1: 20,000 solution, ten minutes later a 1: 5000 solution produced a more marked dilatation and finally a 1: 1000 solution produced a dilatation which continued to increase over a longer period than the preceding, although the first effects were about the same (fig. 12). In no case was there any appreciable effect upon the blood pressure.

In view of the evidence advanced above, that the dorsal root ganglia contain an adrenalin vasodilator mechanism for the hind limb, it was thought possible that there might be a similar one for the intestine and that this might respond to direct application of a solution of adrenalin. With the results of Bayliss (5) in mind we judged that the

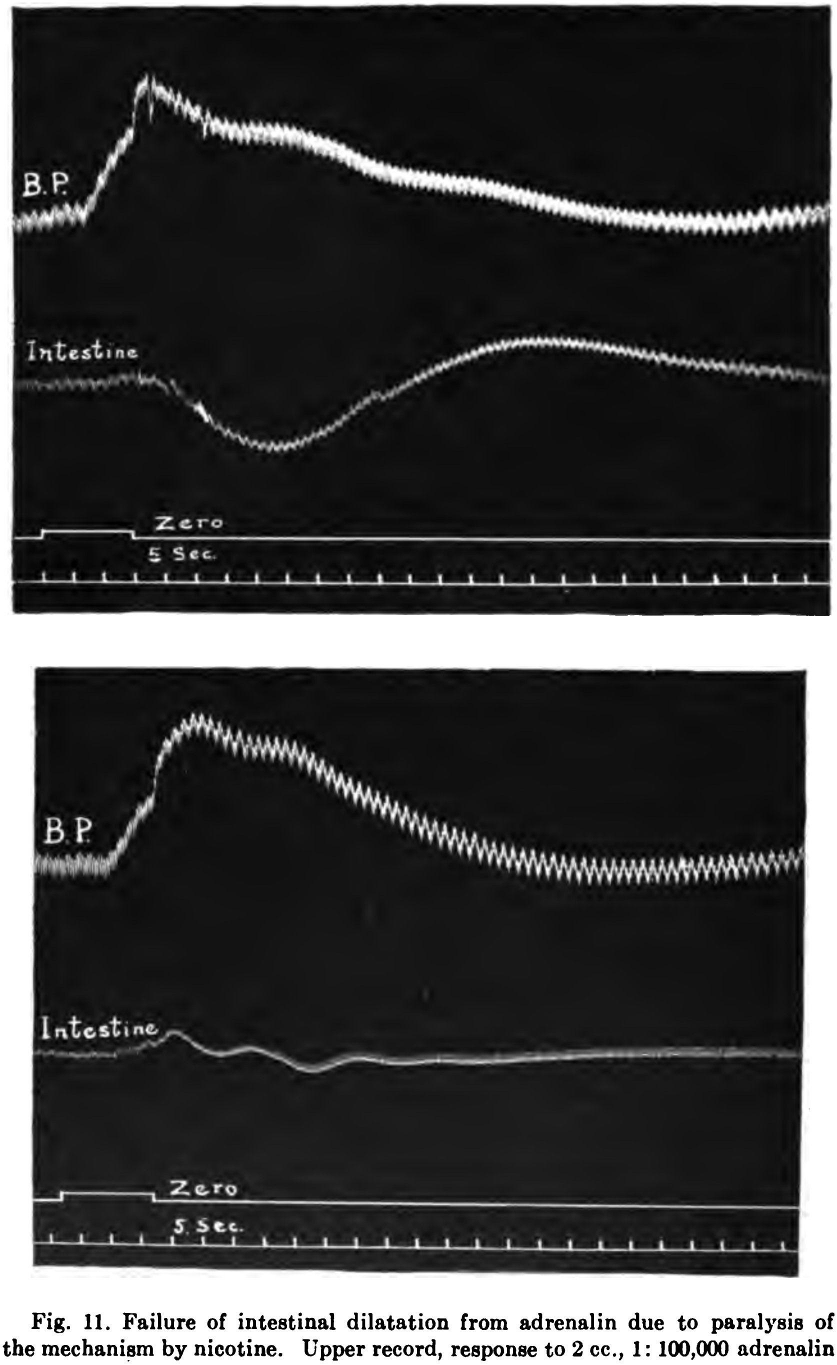

Fig. 11. Failure of intestinal dilatation from adrenalin due to paralysis of the mechanism by nicotine. Upper record, response to 2 cc., 1: 100,000 adrenalin before nicotine. Lower record, response to 2 cc., 1: 100,000 adrenalin after injection of nicotine in the same animal (cat, weight 2.3 kgm.) Base of bellows 20 mm. x 21 mm. (Reduced one-fourth.)

dorsal root ganglia most likely to cause this reaction were those of the twelfth and thirteenth thoracic nerves. We have been able to show that such a mechanism exists, although several of our experiments gave negative results. We investigated seven animals in all, three dogs and four cats, taking records of the volume changes of a loop of intestine on application of a solution of adrenalin 1: 1000 to the ganglia. The preparation of these in the dog was like that described above. In the cat we cut the cord across, drew the cut ends back and got access to the ganglia from their central ends, thus avoiding excessive bleeding. In all cases we found it necessary to cut the ganglia

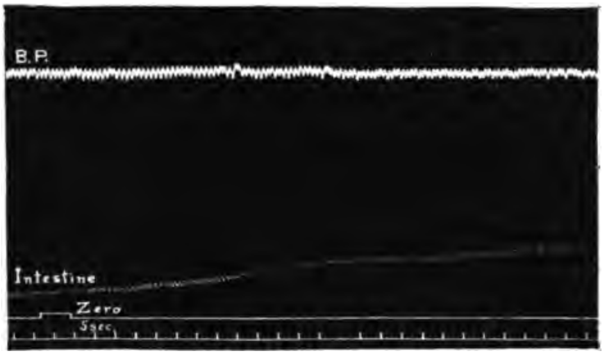

Fig. 12. Dilatation of the intestine due to direct application of 1: 1000 adrenalin to the superior mesenteric ganglion. Cat. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

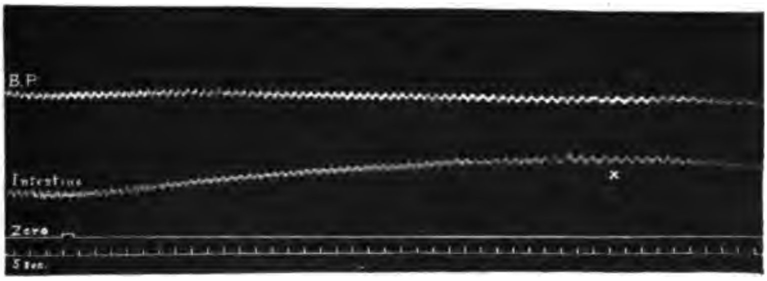

Fig. 13. Dilatation of the intestine caused by application of 1: 1000 adrenalin to a split dorsal root ganglion. Adrenalin washed away with isotonic salt solution at *X*. Cat. Base of bellows 10 mm. x 19 mm. (Reduced three-fifths.)

longitudinally to allow the solution free access to the cells. Of the dogs, one showed no change and the two others only slight dilatations, and these not always occurring. In one case constriction took place. Two of the experiments on cats were more successful. In both the application to the ganglia of three or four drops of the adrenalin solution caused a gradual dilatation of the intestine, which was accompanied by no change or by a slight fall in blood pressure and which gradually disappeared after the ganglia had been washed with saline (fig. 13).

DISCUSSION

It is not surprising to find that the central nervous system does not contain the structures stimulated by adrenalin in bringing about vasodilatation. Cannon and Lyman (6) obtained a fall in blood pressure from adrenalin after total destruction of the central nervous system, if ergotoxine had previously been given. Of course it cannot definitely be said that the central nervous system has nothing to do with the problem, since destruction of portions of the brain or cord inhibits or modifies the response, as is evident in the reversal of blood pressure effects. What our results go to show is that the main seat of the reaction is in the sympathetic and dorsal root ganglia. The same conclusion has been arrived at by widely different ways, viz., 1, by perfusion of the organ, together with destruction or removal of the central nervous system and of one or the other set of ganglia, which might be the seat of the reaction; 2, by the destruction of the ganglia in question, thus preventing the dilatation; 3, by the direct application of adrenalin to the ganglia. The fact that to these ganglia is due the greater part of the dilatation caused by adrenalin does not exclude the possibility of some peripheral action on the dilator nerve endings, as various investigators have suggested, notably Gruber in a recent paper (7). Further research on this question is in progress in this laboratory. At present we are not in a position to say whether the gangliar or the peripheral action is more effective in bringing about dilatation.

The nature of the action of adrenalin on the cells of the sympathetic ganglia is still uncertain, whether it is an inhibition of the constrictor elements or a stimulation of a dilator. The first, in the light of the stimulating action of this hormone on the endings of the fibers from these cells seems improbable. In spite of the negative experiments of Bayliss (5) and others we are inclined to attribute our results to a stimulation of vasodilator cells. If the existence of such cells is a fact, the part, whether cell or synapse, which adrenalin affects, is still uncertain. That it cannot stimulate the fiber directly is evident because no dilatation has ever been obtained in a perfused organ, the ganglia to which have been removed, no matter how few minutes before.

The nature of the adrenalin vasodilator mechanism of the dorsal root ganglia is uncertain, nor is there any evidence as to whether it is similar to that in the sympathetic ganglia. Dogiel (8) has described so-called sympathetic cells in the dorsal root ganglia, but little seems to be known concerning them. Whatever the structure may be, the impulses which are started must be antidromic. From this arises the question of the possible identity of these impulses with those described by Bayliss (9), which brought about dilatation by their action on the vessels of the skin. We have not been able to show conclusively that the dilatation which takes place in a perfused limb, all the nervous connections of which have been destroyed except those with the dorsal root ganglia, is not caused by the vessels of the skin, but all the evidence tends to make it improbable.

SUMMARY

1. Dilatation of the hind limb is brought about by the action of adrenalin on structures located in the sympathetic ganglia of the lower lumbar and sacral regions and in the dorsal root ganglia of the nerves supplying the limb.
2. Dilatation of the intestine is brought about by the action of adrenalin on structures in the superior mesenteric ganglion and in the dorsal root ganglia of the lower thoracic region.
3. Our results tend to support the view that the sympathetic system contains vasodilator fibers to the intestine and to the hind limb.

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XI. THE METABOLIC GRADIENT UNDERLYING INTESTINAL PERISTALSIS

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For many years physiologists have been teaching their students that food goes down the intestine because of Bayliss and Starling's law (1) or Cannon's myenteric reflex (2). According to this law, a stimulus applied to any part of the gut causes a contraction above and a relaxation below. Interesting and important as this law is, it has a number of limitations which, if better known, would undoubtedly have stimulated investigators to pry into the matter a little further or even to look for a new and more universally applicable law. Cannon himself has pointed out that the myenteric reflex is not always in control and that it "does not govern the rhythmic contractions of the small intestine, the rhythmic peristalsis and antiperistalsis of the colon and probably not the rhythmic waves of the stomach." Since then Gaskell (3) has shown that even the word "reflex" may not be strictly applicable in this connection because recent anatomical studies have made it appear very unlikely that there is any nervous arc over which a true reflex could travel.

On reading the few articles that have been written on the subject, one is struck by the fact that the observers found the reflex hard to demonstrate, often absent, sometimes reversed and always localized within 2 to 3 cm. of the stimulated area. They used traumas and, so far as we know, no one has studied the condition of the muscle above and below a bolus of food. Graphic records of peristaltic rushes obtained by Alvarez (4) with six or seven delicate enterographs rarely showed relaxation before oncoming waves. On the contrary, powerful contractions appeared at some distance in advance, and these often succeeded in stopping the rush. A moment's thought will show the need for this; if the reflex were always active, the bowel would soon be emptied. Food once introduced into the duodenum would never stop in its rush to the anus. Another thing which suggests that this law is not the last word and that we should look further, is the complaint of the clinician that it has not been of much help to him in that it does not explain the peculiarities of intestinal action in the sick.

Five years ago, while doing some work on the absorption of gas from the bowels of rabbits and cats, Alvarez noticed that there was a great difference between the irritability of the duodenum or jejunum and that of the lower ileum. When 10 cc. of CO_2 or other gas was injected into the ileum, the bowel would respond with a few contractions, after which it would quiet down; the same amount put into the duodenum or jejunum gave rise to active segmentation, which did not cease until the gas had been absorbed or passed on by a peristaltic rush. Feeling convinced that this difference in irritability alone could account for the downward progress of food, Alvarez attempted to measure it in some way. While trying to do this with excised segments beating in Locke's solution, a gradation in rhythmicity was observed (5). Later, graded differences in latent period were found.

Now, the accepted idea until recently has been that the rhythmic contractions are due to stimuli coming from Auerbach's plexus. This view is based on the work of Magnus (6), who found that strips of longitudinal muscle with the nerve-net adherent would contract rhythmically, while strips of circular muscle without the nerve-net would not beat in Locke's solution. Recently, Gunn and Underhill (7) repeated this work, taking great precautions to avoid trauma, and obtained plexus-free circular strips that would contract rhythmically. This is what we should expect from the fact that some segments of intestine will contract better on the second or third day after excision than on the first. One cannot conceive of nerve cells functioning better after that length of time. Moreover, we know now that smooth muscle cells in cultures will contract rhythmically when there is no question as to the absence of nervous tissue (8).

It would seem, then, that the differences in rhythmicity, irritability and latent period must be ascribed to differences in rate of metabolism in the muscles of the different regions. When we remember that we can take a piece of ileum beating ten times per minute and, by warming it, speed up its metabolism so that it will beat fifteen times per minute, it seems probable that the duodenum, which normally beats fifteen times, has a faster metabolism than that of the ileum. A comparison of the coefficients of increase in rate with increase of temperature in different parts of the small intestine lends support to such a view (9).

Experiments with potassium cyanide. We first attacked the problem with a method which has yielded splendid results in the hands of Child (10). He points out that although oxidation is not the only process taking place in the cell, it may serve as a useful index of the total metabolism. It is pretty well accepted that potassium cyanide interferes with oxidative processes and Child has shown that regions with high rates of metabolism and faster oxidation suffer more from the action of weak solutions of this drug than do regions with slower rates. Thus, if planarian worms of different ages are put into 0.0065 per cent KCN, the younger ones with higher rates of metabolism die before the older ones. If hydras are treated in the same way, those showing the greatest activity die first. In some Ctenophores there is a gradient of susceptibility to KCN in the conducting paths along the rows of swimming plates. The pace-making region suffers so much more than the others that the impulse will sometimes start at the wrong end and the sequence of the beats will be reversed (11).

In doing this work we used five segments of rabbit's intestine in a beaker containing 400 cc. of aerated Locke's solution at 38°C. The animals were killed by a blow on the head and the segments were removed from (1) the upper duodenum (2) the upper jejunum, (3) the upper ileum, (4) the lower ileum and (5) the middle colon. The contractions were all recorded together on the same drum and the segments were all subjected to the same concentration of drug at the same time.

It will be seen from figures 1 and 2 that the addition of 1 part of KCN to 1,300,000 parts of the solution caused a marked loss of tone and rhythmicity in the duodenum and jejunum, while the two segments of ileum were much less affected. We agree with Child that the concentration of the drug must be just right, otherwise the segments will either all stop at once or else they will become acclimated and show nothing. Realizing that a plentiful supply of oxygen might neutralize the effects of the KCN, we stopped aerating the Locke's solution and immediately were able to get better results. Figure 2 shows the marked improvement in the contractions of segments poisoned by KCN when air was passed through the solution. It is plain that the duodenum and jejunum had been suffering much more than had the ileum. Later, when the same dose of KCN was repeated, the first dose still remaining in the beaker, the effects were less pronounced because the oxygen supply was larger.

A large series of experiments was performed next, in which the air current was shut off for varying periods of time, and it was found that with asphyxia alone we could get tracings very similar to those obtained with KCN. Figures 3 and 4 show how much more the duodenum and jejunum suffered from lack of oxygen than did the ileum. This difference was brought out clearly when air was again allowed to bubble

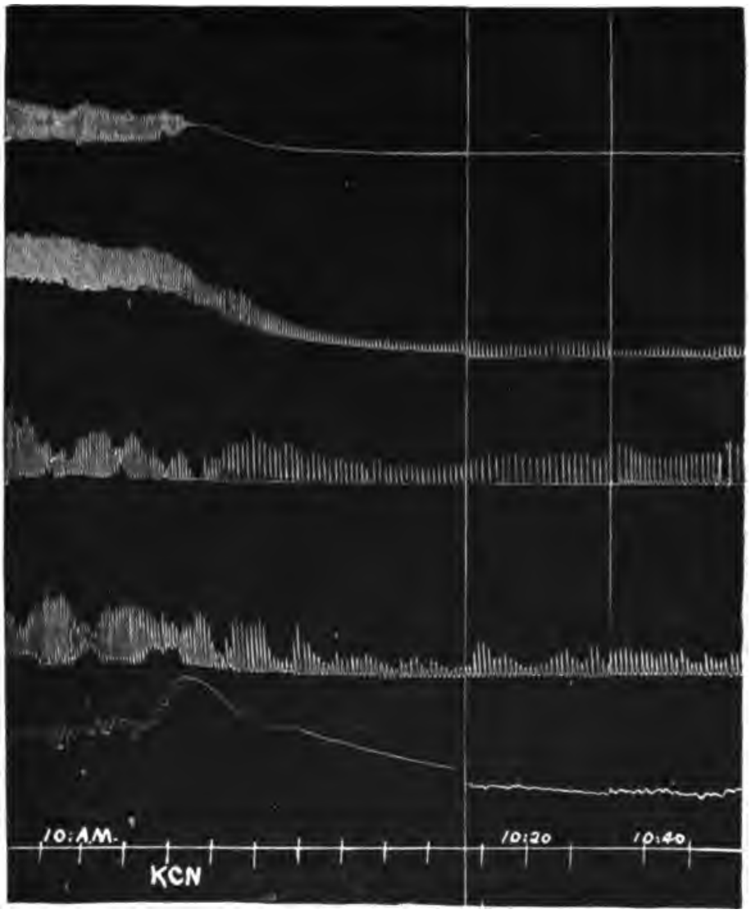

Fig. 1. Graded effects of KCN. From above downwards the segments are from the duodenum, jejunum, upper ileum, lower ileum and colon. Time marking represents minutes.

through the solution. The recovery in amplitude of contraction was then most pronounced in the duodenum.

These results, so comparable with those of Child, furnish considerable proof of the presence of a metabolic gradient from duodenum to ileum. The depression observed in the colon is due probably to other causes and not to a rapid rate of metabolism, since most of our other studies indicate that the colonic muscle is even more sluggish than that of the ileum. It will be noted, on turning to the tracings, that KCN has a slowing effect on the rhythm, probably in addition to and apart

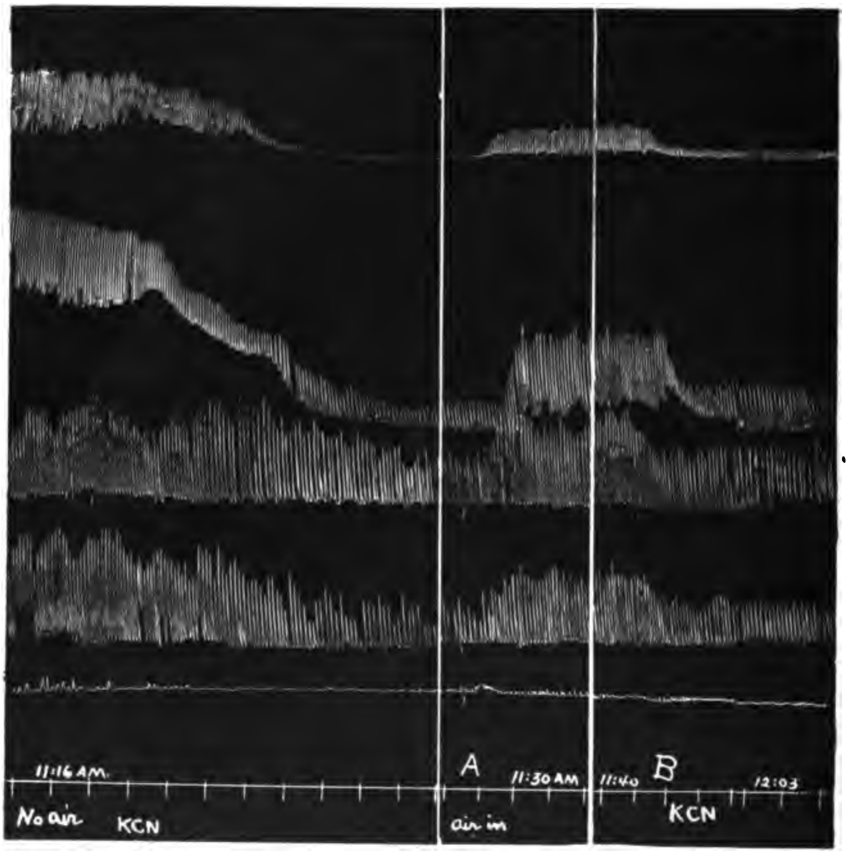

Fig. 2. Effect of KCN on segments in non-oxygenated Locke's solution. At *A*, air was allowed to bubble through the solution; at *B*, more KCN was added.

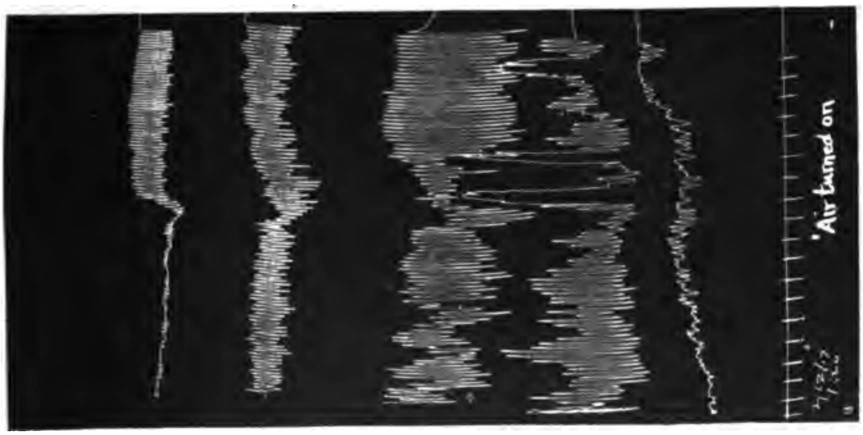

Fig. 3. The effect of asphyxia on segments from different regions.

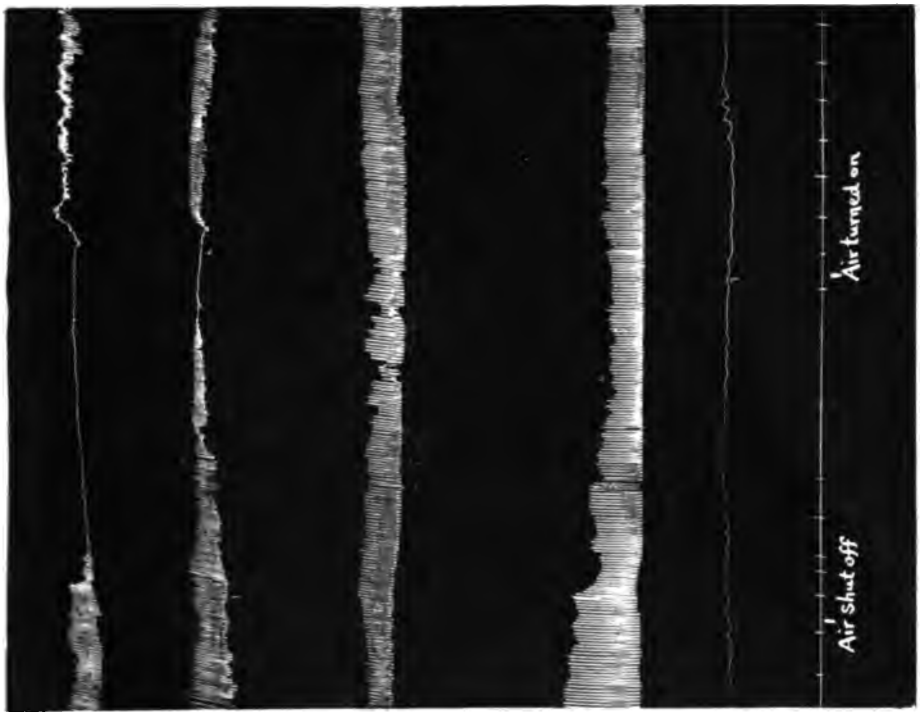

Fig. 4. The effect of asphyxia on segments from different regions.

from its asphyxiating properties. Asphyxia alone does not slow the contractions and although it causes them to disappear in the duodenum, as does KCN, it, unlike KCN, does not affect the colon. In another paper we intend to show that with KCN and other drugs the slowing of the rhythm is most marked in the regions in which the tendency to rhythmic action is weakest. This would explain the marked effect on the colon since those segments are the hardest to start beating in the Locke's solution.

Experiments with adrenalin. The objection may be raised that perhaps five such segments will react to all depressant influences in this graded way and that metabolism has nothing to do with it. After testing some seventy-five drugs, we can say that not only do many of them affect the segments equally but some, such as adrenalin, will often show a beautiful gradient in the opposite direction, that is, the duodenum will be very slightly affected when the ileum is completely paralyzed. This graded effect with adrenalin may easily be due to the same gradiation in intensity of oxidation which we believe produces the differences with KCN. It is well known that adrenalin in dilute solution is rapidly oxidized to an inert substance. If our theory is correct, this change would naturally be brought about soonest in the duodenum and that segment would be the soonest to escape from the influence of the drug. A glance at figure 5 will show that that is what actually happens. With smaller doses, tracings were obtained in which the ileum was seen to suffer considerably in amplitude while the duodenum was unaffected. Apparently the drug was oxidized before it could reach its seat of action in the muscle. Figure 6 shows that these differences are not due to a higher threshold for drug action in the duodenum because immediately following a characteristic adrenalin action, some apocodein produced its most marked effect on the duodenum. In discussing this subject with some physiologists, the objection was raised that these differences should be ascribed to some peculiarity in sympathetic nerve supply. It seems to us that until such complicated differences are clearly understood it is better to rely on the simpler chemical explanation just given.

Measurement of carbon dioxide production. We next attempted to measure the amount of CO_2 given off by the different segments in a unit of time. The technic used was suggested to us by Doctor Van Slyke, to whom our thanks are extended. Weighed segments of rabbit's, cat's and white rat's intestine were put into a large test tube containing Locke's solution at 38°C. In most of the experiments the

0.02 per cent $NaHCO_3$ of the Locke's solution was replaced by 0.01 per cent NaOH although, as was to be expected from the small amount of carbonate present, any error introduced in this way was practically negligible. Oxygen was allowed to bubble through the solution and then over through two other tubes, each containing 50 cc. of n/5 $Ba(OH)_2$ solution. We agree with Fletcher (12), who used a similar technic for studies with striated muscle, that these two tubes are sufficient to catch all the CO_2 carried over. At the end of an hour the

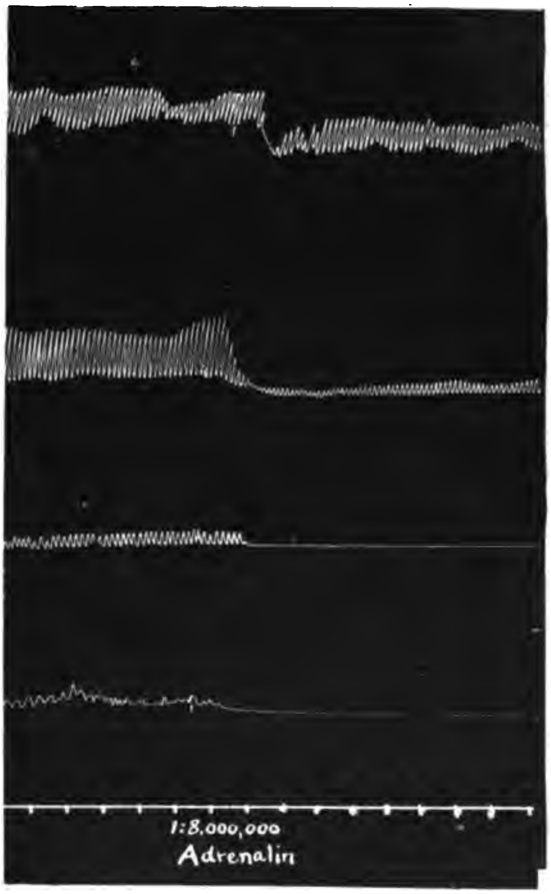

Fig. 5. The graded effect of adrenalin. From above downwards, the segments are from duodenum, upper ileum, lower ileum and colon.

two barium hydrate tubes were titrated as rapidly as possible with n/5 HCl. In practically all of these experiments the difference between the CO_2 production of duodenum and ileum was quite marked and was according to expectations. Following are some of the protocols. The figures represent cubic centimeters of n/5 $Ba(OH)_2$ neutralized by CO_2. They vary in the different experiments because different weights and different times were used.

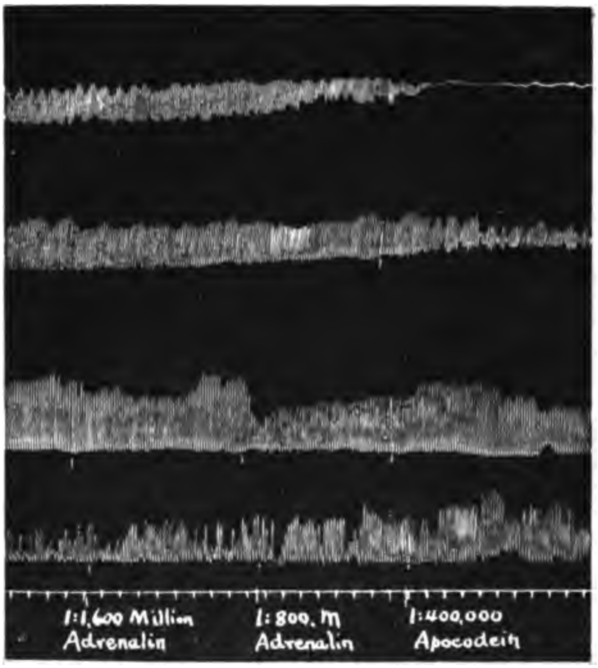

Fig. 6. Graded effects of adrenalin and apocodein. From above downward the segments are from duodenum, jejunum, upper ileum and lower ileum.

	RAT	RAT	RAT	RAT	RAT	CAT
Duodenum	8.5	5.6	9.4	17.4	25.4	20.0
Jejunum				17.0	19.7	15.0
Middle				16.0	10.5	18.0
Ileum	7.8	3.2	7.0	12.0	21.0	15.0

Here, as in all subsequent experiments, poor results were to be expected when some segments contracted more actively or more constantly than others. Good results were obtained either when they all remained quiet or when they all became equally active. It can easily be seen that a very active ileum might give off as much CO_2 as a quiet duodenum. As the segments do not all recover from trauma and start beating at the same time, it was found well in all these experiments to leave the pieces of bowel in warm oxygenated Locke's solution for half an hour after cutting and weighing. When all were going well, they could be transferred gently to the test tubes. Another source of error undoubtedly arises in the fact that so many of the animals have intestinal parasites, i.e., coccidiosus and trichocephalus in the rabbit, round worms and taenias in the cat. Alvarez has pointed out elsewhere that these conditions tend to flatten or even reverse the gradients of rhythmicity and latent period found in normal animals (13). For this reason it is best to give cats and dogs a vermifuge sometime before they are to be used.

Much better results were obtained with the very simple and exceedingly delicate method of Haas (14). Small segments of rat's intestine were opened out, washed thoroughly with Locke's solution, blotted between filter papers and cut to some arbitrary weight, usually 0.4 gram. For purposes of control, two segments were taken from each of the locations described above. After their recovery from this trauma they were transferred to thick, glass Wassermann tubes containing Locke's solution to which 1 part in 20 of a 0.01 per cent watery phenolsulphonephthalein solution was added. The tubes all held about the same amount of solution. They were sealed with corks which had been boiled in paraffin and, in addition, the ends of the tubes, corks and all were paraffined. Only a small bubble of air was left in the tube. These tubes were left in a water bath at 38°C. until the CO_2 formed had brought about a considerable decolorization of the solution. Haas has shown that this method will detect differences in hydrogen ion concentration as small as 1×10^{-5}.

Ordinarily the solution about the duodenal segment would begin to fade within a few minutes and in half an hour the whole tube would often be acid in reaction.

As soon as this decolorization was complete in the tubes containing the duodenum, they were all removed from the bath and arranged against a white background according to the intensity in color. The tubes containing the colonic segments generally retained most of their original pink tint. After the tubes were graded, the labels on the corks were read and put down as in the following protocols. It will be seen that although at times, probably on account of unequal activity, the controls are somewhat separated, on the whole the gradation is according to expectation. D represents duodenum; J, jejunum; M, middle; I, ileum and C, colon.

Guinea pig	*White rat*
D J D J M M I I C C	D D J J C M M I I C
D D J M J I I M C C	D D J M J M I I C C
D J D J M M I I C C	D D J J M I I M C C
D D J J M M C I I C	D D J J M M I I C C
D D J J M M I I C C	D D J J C M M I I C
Rabbit	*Cat—muscle alone*
D D J J M I C M I C	D D J C J M M C I I
J D D J M I I M C C	D D J J M I I M
D D J J M I M I C C	D J M I C
D J M D M J I I C C	D J M I C
D D J J M I M I C C	

The results with the white rats and guinea pigs were best, perhaps because their intestines are smaller; longer segments can be used and the proportion of cut surface to segment is much less than in rabbits and cats. It must also be remembered that, with this technic, the Locke's solution is not aerated and we have just seen that lack of oxygen interferes more with the activity of D and J than with that of M, I and C. This difference would tend to obscure the differences in CO_2 production that have been found. The muscle from the cat rarely showed much activity, often none at all.

In order to get some idea of the actual amounts of CO_2 formed, the tubes were opened and titrated rapidly back to the original color with n/200 NaOH. As was to be expected, what with losses of CO_2 and difficulties in getting the end point, the gradation obtained was not so satisfactory as with the closed tubes. In seven experiments, small amounts of the solution were removed and the CO_2 measured in the Van Slyke apparatus. In the following protocol the figures represent cubic centimeters of n/200 NaOH.

Rat (1 hour)

D	D	J	M	J	M	I	I	C	C
5.6	3.8	2.8	2.6	2.4	2.2	1.6	1.2	1.2	1.2

The average amounts in five experiments were

D	J	M	I	C
3.1	2.7	2.5	1.9	1.6

In the following experiments in which the Van Slyke apparatus was used, the figures represent cubic centimeters of CO_2 in 1 cc. of the solution.

Guinea pig

D	J	M	I	C
0.49	0.22	0.18	0.16	0.14

Guinea pig

D	J	D	J	I	I	M	M	C	C
0.30	0.26	0.24	0.21	0.20	0.20	0.17	0.16	0.16	0.02

The average amounts in seven experiments were

D	J	M	I	C
0.30	0.23	0.19	0.18	0.16

The differences between some of the controls in these experiments again show the need for having all of the segments beating well.

The fact that the faded tubes would return to their original color when oxygen was bubbled through them showed that the decolorization was due to CO_2 and not to some other acid.

In order that the bowel might be studied while contracting actively, most of the experiments were done at first with segments containing mucous membrane. As this membrane varies in thickness in different parts of the gut, segments of equal weight might contain different amounts of muscle. An error would creep in also on account of the formation of CO_2 in the mucosa. The fact that decolorization was very slow in tubes containing inactive segments indicates that this error was not large. Moreover, some six experiments done so far show that the CO_2 production in the mucosa is also graded from duodenum to ileum. To rule out all objections, strips of muscle peeled off from the mucous membrane of the cat's intestine were studied with the same methods, and the same graded production of CO_2 was found.

Tests for oxidases and peroxidases. We next attempted to show a gradation in the oxidase and peroxidase content of the muscle from different regions. Preliminary qualitative tests, however, failed to show a measurable amount of these substances in any part of the gut. With or without the addition of H_2O_2, there was practically no bluing of guaiac or benzidin with the crushed muscle. The activity of the reagents was controlled with a little potato juice and with the mucous membrane of the small intestine. Experiments with pyrogallol, hydroquinone, pyrocatechin and metol, using Bunzel's small apparatus (15) showed practically no oxidation on prolonged contact.

There are many reasons why such experiments should be disappointing. We have not as yet identified the ferments, if such there be, which help the tissues to burn their proper fuels,—sugar and fat; and these more or less specialized oxidases which have been identified are probably of lesser importance. Moreover, as Loew has pointed out, the living state of protoplasm seems to be the most important thing in cell oxidations because otherwise, heating to a temperature of 45°, which kills the tissue and does not destroy the oxidases, ought not to interfere with respiration in the way it does.

Catalase estimations. More and more evidence is accumulating to show that the catalase content of a tissue is a better index to its metabolic activity than is its oxidase content. Those who are interested can get an introduction to this literature through the articles of Batelli and Stern (16), Loew (17), Kastle (18), Zieger (19) and Zaleski and Rosenberg (20). In spite of the large amount of work done upon this substance and in spite of the fact that it is found in considerable amounts in most animal and vegetable cells, its biologic significance is still far from clear. Usher and Priestly (21) have shown that in plants H_2O_2 is one of the substances formed during the action of light on CO_2. If not removed immediately by the catalase it bleaches the chlorophyl and puts an end to the photosynthetic reactions. Many believe that catalase has a similar protective function in animal tissues. So far, most of the evidence indicates that it is a highly specific ferment acting on hydrogen peroxide alone. Ewald (22), however, has offered considerable proof for his theory that catalase helps in tissue respiration by loosening the oxygen from oxyhemoglobin. He found that the reduction of oxyhemoglobin in ammonium sulphate solutions takes place faster in the presence of catalase. This is the more suggestive in view of Peters' (23) finding that the amount of oxygen taken up by hemoglobin corresponds to that required to convert its iron into a peroxide. Fischer and Brieger (24) have also shown that the blood oxygen may be held in the form of a peroxide.

It seems probable that in some way the catalase assists in furnishing oxygen to the tissues as fast as they require it. Although there are a number of exceptions still to be explained there is, on the whole, a pretty definite relation between the metabolic activity of a tissue or of an organ and its catalase content. Thus Lesser points out that the largely anaerobic ascaris lumbricoides has one forty-eighth of the catalase content of the aerobic earthworm (25). The catalase content of bacteria has a close relation to their use of oxygen (26). As a general rule, the sum of the catalase content of blood and liver is less in cold-blooded animals than in warm. There are some exceptions which may be explained by future work. For instance, the blood of a bird has very little catalase although its respiration is very active. This may be due to the derivation of birds from reptilia; and it may be that other tissues make up for the deficiency. Thus it has just been shown that the catalase content of a chicken's skin is higher than that of any other skin studied (27). Other objections have been raised by Amberg and Winternitz (28) who showed that although the fertilization of sea-urchin's eggs leads to an increase of from four to six times their cell oxidation, there is no increase in catalase. Zieger (19), however, showed an increase in catalase content of insects during their metamorphosis to the pupal stage. The conflicting results obtained by some of the workers may easily have been due to their neglect of certain factors modifying the speed of the reaction.

Appleman (29) has shown in potatoes that the catalase content is a better index of respiratory activity than the oxidase content. The catalase rises and falls with the CO_2 production while the oxidase does not. Zaleski and Rosenberg (20) while pointing out a number of objections, conclude from a review of the literature and their experiments with germinating seeds that "the oxidase and catalase effects represent the common function of one and the same substance or complex." Loevenhart and Kastle (30) conclude from parallel experiments with formic acid and H_2O_2 that "in proportion as a substance is able to break down the peroxide, so also is it able to accelerate oxidations." Burge (31) feels sure from some experiments with the muscles of exercised and confined animals that catalase is an index of the metabolic activity of a tissue. Doctor Child permits us to state that work done so far in his laboratory by Mr. MacArthur indicates that the catalase content of different parts of small animals follows the gradients of KCN susceptibility and CO_2 production previously established. Zieger also comments on the fact that younger organisms, with more rapid metabolism, have larger amounts of catalase.

Fortunately, the methods for estimating catalase are very simple. The tissue to be tested is weighed and ground up in a mortar with broken glass. The grinding should be done uniformly or the results will vary. In one experiment the finely ground tissue liberated ten times as much H_2O_2 as did the expressed juice. The glass and tissue are washed into a large test tube with 15 cc. of water, and 15 cc. of 3 per cent hydrogen peroxide is added. This is shaken for fifteen minutes, and the oxygen given off is measured as it displaces water in a burette. The readings are made at atmospheric pressure. We have found it best to run five tests at a time, using a shaking machine. This not only saves a great deal of time, but it eliminates three considerable sources of error, i.e., differences in the temperature of the room; marked differences in the temperature of the tube, depending on the extent and duration of contact with the shaker's hand; and differences in the amount of shaking. Even with the shaking machine, all the tubes must be held firmly in a block so that some will not be agitated more than others. Another possible source of error avoided by doing the tests together is an unequal lighting. Fortunately, the destructive effect of light on the ferment is slight in the first fifteen minutes, during which time most workers make their determinations. After that, however, surprising differences may be obtained according as the tests are done near to or far from a window (32). When all is ready, the H_2O_2 is run in from a thistle tube, the top of which is connected to one arm of a Y. Another arm connects with the test tube and the other with the burette. Most of our experiments had been done before we read the papers of Loevenhart (33), McGuigan (34), Mendel and Leavenworth (35) and Issajew (36), all emphasizing the importance of neutralizing the hydrogen peroxide before using. We have repeated enough of the work to show that for our purposes it does not make any difference whether this is done or not. The actual amounts of gas given off are larger with the neutral hydrogen peroxide but the gradations to be described later remain the same. The increase is slight for the muscle catalase and more marked with the mucous membrane. To control the technic, a number of tests were run on strips of muscle from the same segment of gut or from adjoining segments, and the error was found ordinarily to be between 1 and 2 per cent.

When segments from different levels were studied, the amounts of oxygen were found to be graded much as the CO_2 was graded. This will be seen from the following figures.

Cat muscle

	PEROXIDE NOT NEUTRALIZED									AVERAGE
Duodenum	21.8	22.5	23.2	22.0	41.0	25.6	39.7	31.7	24.2	27.9
Jejunum...	20.5	20.5	20.3	18.1	35.5	28.0	23.2	24.5	22.7	23.7
Middle.....	17.1	20.2	18.1	16.6	36.7	22.4	26.2	26.7	13.4	21.9
Ileum......	6.0	13.2	13.5	15.8	34.6	20.5	17.6	18.5	11.7	16.8
Colon......	9.9	15.0	13.7	18.4	27.8	22.3	19.3	23.3	14.9	18.3

Dog muscle

	H_2O_2 ACID		H_2O_2 NEUTRAL (PUPPY)		AVERAGE
Duodenum	15.7	14.3	18.3	27.9	19.0
Jejunum	13.2	14.3	17.9	26.6	18.0
Middle	11.9	12.5	13.6	26.0	16.0
Ileum	11.3	12.5	12.6	21.1	14.4
Colon	11.2	11.1	17.0	22.2	15.4

In all these experiments 0.3 gram of tissue was used. The figures represent cubic centimeters of gas evolved after fifteen minutes. In the cat and dog one can easily peel the muscle off the mucous membrane and get it clean except for the thin layer of peritoneum. This cannot be done with the thin bowels of rabbits and rats, so one must there be content with scraping off the mucosa. For some reason or other, this can be done more easily in the duodenum and colon than in the jejunum and ileum.

Rabbit muscle from which the mucosa has been removed by scraping

	H_2O_2 NEUTRALIZED						AVERAGE
Duodenum	42.2	37.2	33.4	36.9	41.5	39.6	38.5
Jejunum	37.8	32.8	27.7	35.6	33.5	32.7	33.4
Middle	39.7	34.2	27.3	24.8	32.4	35.7	32.4
Ileum	32.8	36.7	20.4	31.2	30.0	33.4	30.8
Colon	27.1	29.0	21.4	15.9	21.0	16.8	20.9

It will be seen that the gradient is the same as in the cat. In a number of pregnant and diseased rabbits and in one apparently normal animal, the duodenal figure was lower than that of the jejunum. A similar difference was observed while measuring the latent periods in distempered dogs. The duodenum seems always to be the first to suffer from a general intoxication or from adverse conditions.

As the catalase content of the mucous membrane was found to be graded also from duodenum to colon, the following figures from rabbit and rat may be assumed to represent the sum of graded muscle catalase and graded mucous membrane catalase.

Rabbit muscle with mucosa

	H_2O_2 NOT NEUTRALIZED						AVERAGE
Duodenum	46.8	49.4	47.0	52.8	40.3	51.0	47.9
Jejunum	41.2	44.7	49.8	34.8	38.5	45.5	42.4
Middle	32.3	43.3	44.2	36.2	36.4	39.2	38.6
Ileum	24.8	34.3	39.0	32.4	29.7	19.8	30.0
Colon	14.2	21.9	15.6	21.1	20.8	8.2	16.9

White rat muscle with mucosa

	H_2O_2 NOT NEUTRALIZED							AVERAGE
Duodenum	28.2	18.1	21.2	21.6	30.2	19.5	25.5	23.5
Jejunum	27.2	22.0	13.2	21.2	27.2	19.5	22.2	21.8
Middle	23.3	13.7	13.0	20.8	26.0	19.0	21.4	19.6
Ileum	19.2	12.9	14.0	21.2	26.4	15.7	19.2	18.4
Colon	20.0	6.0	9.8	18.0	22.8	18.3	17.2	16.0

Since in this work the essential thing is the gradient observed in the different sets of five determinations, made at the same time under identical conditions, and not the absolute values of O_2, we have not bothered to reduce the figures to a common temperature and pressure. There are often marked individual differences in the catalase content of muscle from the same region in different animals. The fact that the cat muscle contracts firmly to a pearly white cylinder which does not give the benzidin test shows that the graded differences found are not due to differences in blood content. We reserve for another paper a discussion of similar gradients observed in the catalase content of muscle taken from different parts of stomach and colon. Interesting variations have been found also in the gradient of catalase content in intestines from sick and pregnant animals.

DISCUSSION

It is our belief that the gradient of metabolism shown by these various tests is the underlying basis of downward peristalsis. The impulse in the heart has long been known to follow a gradient of rhythmicity. Miss Hyman, in Child's laboratory, is finding, as was to be expected, that there is a gradient of metabolism underlying the differences in rhythmicity. When we speak of the negativity of the sinus region to other parts of the heart, we are thinking of the direction of current flow through the galvanometer; on the heart side of the circuit, the sinus region is really positive to other parts, indicating that it has the highest rate of metabolism. Recently Tashiro, with his marvelously sensitive biometer, has demonstrated a gradient of CO_2 production in nerves, and there seems little doubt but that the nerve impulse flows along that gradient (37). In an efferent nerve the gradient is from the center to the periphery; in an afferent nerve the peripheral end has the greater CO_2 production and the gradient runs toward the center. Similarly we believe that the intestinal contents move aborally because of the aboral gradient of metabolism in the muscle. Scientific men who have been trained by physicists, chemists, electricians and irrigation engineers to expect motion only in the presence of differences in potential or force should find no difficulty in accepting such a theory.

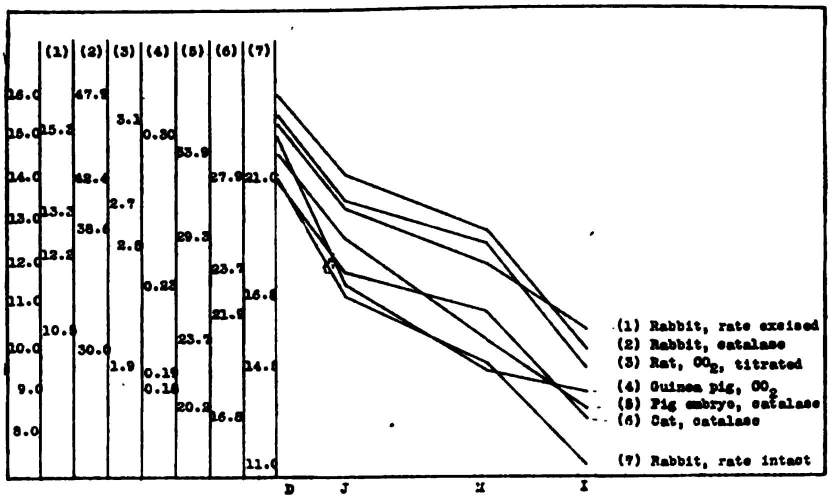

Fig. 7. Showing the parallelism between the gradients of rhythmic contraction, CO_2 production and catalase content in different animals. In order to bring the different curves closer together, an arbitrary set of ordinates was chosen running from 8 to 16. The seven sets of data were then multiplied or divided by factors which would place the first figure for the duodenum between 14 and 16. The original data are shown as ordinates in the seven columns on the left. The numbered columns correspond to the numbered legends identifying the different curves. The abscissae represent the four segments along the gut.

Some may object that we have put the cart before the horse and that a greater amount of CO_2 is formed in the duodenum simply because it beats oftener and does more work. They may point to the fact that poor results were obtained in the CO_2 studies when the segments did not all contract well. To meet this objection we repeated the work with strips of muscle which either did not contract of themselves or else were paralyzed with adrenalin, and we obtained the same graded results. This is what we should expect, also, from the catalase experiments, which show chemical differences in the minced and quiet muscle. Some may still maintain that these differences are brought about by differences in function. Child has aptly compared the life processes to a river whose course is directed by banks which, in their turn, are moulded by the river. We believe that the "banks" just discovered are responsible for the "river;" others may feel that the "river" is still unexplained and that the "banks" are just those which one should expect to find carved by such a stream. It must be remembered, however, that most of the carving takes place over thousands of years during phylogenetic development, and that in any one member of the race these banks are fairly rigid. It seemed well, nevertheless, to follow a suggestion made by Doctor Whipple that we study the intestine in embryos, where function (if any peristalsis attends the formation of meconium, it must be insignificant as compared with that during extrauterine life) has not yet commenced. In terms of Child's simile, let us see whether the "banks" are there before the (individual) "river" has begun to flow.

The following figures represent the catalase value for the muscle (mucous membrane scraped off) from different parts of the intestines of 20 cm. pig fetuses.

	H_2O_2 NEUTRALIZED					AVERAGE
Duodenum	40.6	38.7	28.5	29.9	27.6	33.1
Jejunum	39.9	35.8	20.5	27.2	23.1	29.3
Middle	25.0	25.5	17.2	27.4	23.5	23.7
Ileum	17.0	20.1	16.2	23.6	23.9	20.2
Colon	11.7	15.2	10.6	16.5	18.3	14.5

It will be seen that here again we have the same pronounced gradient from the duodenum to the colon that has been found in adult animals.

Fortunately, we have at our disposal some other experiments which show that the gradient of activity will remain unchanged even when, for many months, the ileum is made to serve as jejunum and the jejunum as ileum. A number of men have reversed long stretches of intestine in dogs and have kept the animals alive for a year or more on a perfectly smooth diet (38). Finally, however, all the dogs died with symptoms of intestinal obstruction, which at autopsy was shown to be due to the accumulation of wisps of straw, bits of bone and other rough material (surreptitiously obtained) just orad to the upper suture. This observation, together with a number of others, convinced the experimenters that the direction of peristalsis had remained unchanged. Although fluids could be forced "uphill" through the reversed bowel, solids could not.

Those who may still feel that the greater activity of the duodenum accounts for its more rapid metabolism have yet to explain the origin of the greater activity. What calls it forth and directs it if it is not some peculiarity of the local musculature? They cannot push the thing off into some ganglion or other because the peculiarities are observed in excised strips of muscle. They can hardly ascribe it to impulses from nerve cells in Auerbach's plexus because the peculiarities persist for three or four days after excision.

If a man designing a cannery wished to convey the cans on a series of belts at varying rates through a number of cooking vats, he would not depend on the intelligence of the cans or upon their desire to linger over some of the processes; he, himself, would regulate the speeds of the different belts to suit the different needs. Similarly, it seems to us that if any one could conceivedly design a bowel in which food and ferments were to be mixed in proper proportions and carried along at rates varying with the needs of absorption, he could hardly do better than to place muscles with a higher metabolic rate, greater rhythmicity, etc., at those points where rapid movement was desired.

These ideas may seem strange to any one who thinks of smooth muscle as an entity, but we feel sure that if such a person were to spend a few months getting records of rhythmic contraction from segments from different parts of the gut, studying the irritability, tone, latent period, form of contraction curve, susceptibility to trauma and disease toxins, and the reactions of rate, rhythmicity and tone to drugs, he would be satisfied that he had been dealing in the different regions with different muscles suited to the different functions. Thus, the feces could not lie quietly in the cecum or colon if the muscles there were as active and as responsive to stimuli as they are in the duodenum. This brings up another point: that the speed with which the intestinal contents are forwarded depends, probably, not only on the steepness of the gradient but also upon other characteristics of the muscle.

Changes in the gradient of metabolism with symptoms of indigestion might be brought about (1) by a general depression of the body strength or by a general bacterial intoxication which would affect the duodenum more than the ileum; (2) by chronic passive congestion, as in heart disease, the duodenum suffering most from the poor oxygen supply; (3) by a local increase of blood supply, such as probably occurs in the colon in the presence of an inflamed, pregnant or menstruating uterus, and (4) by inflammations, such as appendicitis, which raise the local metabolism above its proper level. In two recent papers, Alvarez (39) has gone over a large number of clinical and radiological observations and has shown how beautifully they fit into such a theory.

Incidentally, those who are interested in the biologic significance of catalase, will find in the parallelism of the curves of rhythmicity, CO_2 production and oxygen liberation in figure 7, added proof of the close relation of this substance to metabolism.

SUMMARY

The "Law of the Intestine" has so many limitations that we should look for an additional or underlying cause for downward peristalsis.

Gradients of rhythmicity, irritability and latent period have been demonstrated in the intestinal muscle from duodenum to colon. All the evidence now points to a myogenic origin for the rhythmic movements.

Five segments of intestine contracting rhythmically in Locke's solution show a graded susceptibility to low concentrations of KCN, the duodenum suffering most. A similar graded effect can be obtained with asphyxia. There is considerable evidence to prove that such gradients of KCN susceptibility and asphyxiation correspond to gradients of oxidative activity.

The graded response of the segments to adrenalin is explained on the same basis. The more rapid oxidation of the drug entering the duodenal wall enables that segment to escape promptly from its action.

Using two different methods, it has been shown that, per unit of weight, there is a graded production of CO_2 both in the muscle and in

the mucous membrane from duodenum to colon. This gradient was observed even when the muscle was kept paralyzed by adrenalin.

No measurable amounts of oxidase or peroxidase could be found in the muscle. A peroxidase is present in the mucous membrane of the small intestine.

The catalase content of muscle and of mucous membrane per unit of weight is found to be graded from duodenum to ileum.

These observations all point to the presence of a metabolic gradient in the muscle, a gradient which the writers believe underlies and gives rise to the gradients of rhythmicity, irritability and latent period. They believe that these gradients determine the direction of peristalsis just as similar gradients direct the impulse in the heart.

Many disease conditions can be explained best on the basis of upsets or differences in steepness in these gradients.

Added proof is given for the view that the catalase content of a tissue is an index to its metabolic activity.

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SECRETIN

III. Its Mode of Action in Producing an Increase in the Number of Corpuscles in the Circulating Blood

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In previous reports (1), (2) we have pointed out that secretin is capable of producing a considerable increase in the number of erythrocytes and leucocytes in the circulating blood. We have further suggested that the increase is due to an increased production of blood corpuscles probably by direct stimulation of both the bone marrow and the lymph glands. If this be true its repeated administration over a long period of time should effect definite changes in the blood picture and, in the organs, the histological changes of increased activity.

In accordance with this idea secretin was given hypodermatically to two rabbits to a total of forty doses. The preparation of secretin employed was a dried acid extract as in our previous experiments (2), and the dose of 10 mgm. per kilogram of body weight was dissolved in 2 cc. of physiological saline solution. To each of two control rabbits 2 cc. of physiological saline solution were administered subcutaneously at the same time and under the same conditions. The injections were made every day for two weeks, three times a week for the next two weeks, then every day for the third two weeks, and finally three times a week for two weeks. Thus the entire time during which the experiments were conducted was eight weeks, from December 10, 1917 to February 4, 1918. Each pair of rabbits comprised a male and a female and for convenience will be referred to hereafter as the secretin rabbits, nos. 1 and 2, and the control rabbits, nos. 3 and 4. They were fed and cared for under our personal supervision. We wish to emphasize particularly that there was at no time evidence of infection in any of the rabbits, nor did either of the females become pregnant. Their appearance remained perfectly normal and all showed satisfactory gain in weight, the average for the four animals being 257 grams during the time under observation.

Daily at first but somewhat less frequently later in the course of the experiments, prior to giving the injections, blood was withdrawn from the ear of each rabbit for counting both the red and white corpuscles. The counts were made early in the morning before the animals were fed. Physiological saline solution and 0.5 per cent acetic acid were used as diluting fluids for the red and white corpuscles respectively and the counts were made in the usual manner with the Thoma-Zeiss apparatus. Blood smears were obtained at the same time, subsequently stained and differential leucocyte counts made, the results of which will be detailed later.

Table 1 gives the initial erythrocyte and leucocyte counts in each of the secretin rabbits made before the first injection and the counts week by week, these latter being in each instance the average of all the counts made on that particular animal during the week. Table 2 gives the corresponding counts for the control rabbits. At the bottom of each table is also given the percentage relation of the various counts, considering the initial count as 100 in each case.

A considerable increase in the leucocyte count was promptly produced in the secretin rabbits. This amounted to 52.52 per cent in the first week; in the third week the increase was 146.69 per cent, and even in the eighth week the total leucocyte count was still 37.56 per cent above the initial count in these rabbits. The control rabbits, meanwhile, showed comparatively slight daily variations in the leucocyte count. On two occasions the weekly average was as much as 20 per cent above the initial count but these high counts were evidently only manifestations of the variation that normally occurs in the number of white corpuscles in the rabbit. The leucocytes in the control rabbits did not show the same great and persistent increase in number that was apparent in the secretin rabbits.

Less pronounced change in the erythrocyte count was effected though a comparison of the two tables permits the observation that, while the initial count in the control rabbits was higher than in the secretin rabbits, at the end of the experiment the count in the secretin rabbits was not only relatively greater, 18.47 per cent, but also absolutely higher. We have observed before that the erythrocyte count in normal rabbits may increase to some extent during long periods under observation probably because the living conditions, food, etc., are improved. The increase in the count in the secretin rabbits, however, is greater and occurred more promptly than any effect we have ever observed in normal animals as the result of environmental improvement alone.

TABLE 1

Erythrocyte and leucocyte counts in secretin rabbits

NUMBER		INITIAL COUNT	FIRST WEEK AVERAGE	SECOND WEEK AVERAGE	THIRD WEEK AVERAGE	FOURTH WEEK AVERAGE	FIFTH WEEK AVERAGE	SIXTH WEEK AVERAGE	SEVENTH WEEK AVERAGE	EIGHTH WEEK AVERAGE
1	W. B. C.	9,300	17,183	22,166	22,266	25,833	29,300	16,250	17,400	13,100
	R. B. C.	6,608,000	5,929,000	4,703,000	6,150,000	5,178,000	6,858,000	6,300,000	6,944,000	7,562,000
2	W. B. C.	10,400	12,866	12,916	26,133	20,333	13,000	14,200	16,950	14,000
	R. B. C.	4,432,000	4,941,000	5,208,000	6,150,000	5,042,000	6,715,000	6,336,000	7,196,000	7,332,000
Averages....	W. B. C.	9,850	15,024	17,541	24,199	22,933	21,150	15,125	17,150	13,550
	R. B. C.	5,520,000	5,435,000	4,955,500	6,150,000	5,110,000	6,786,500	6,318,000	7,070,000	7,447,000
Percentage relations	W. B. C.	100.00	152.52	178.08	246.69	232.82	214.63	153.55	174.03	137.56
	R. B. C.	100.00	98.46	89.77	111.41	92.57	122.94	114.45	128.07	134.90

TABLE 2

Erythrocyte and leucocyte counts in control rabbits

NUMBER		INITIAL COUNT	FIRST WEEK AVERAGE	SECOND WEEK AVERAGE	THIRD WEEK AVERAGE	FOURTH WEEK AVERAGE	FIFTH WEEK AVERAGE	SIXTH WEEK AVERAGE	SEVENTH WEEK AVERAGE	EIGHTH WEEK AVERAGE
3	W. B. C.	7,100	7,833	7,166	11,800	7,833	7,600	6,800	9,000	8,533
	R. B. C.	6,272,000	5,536,000	5,784,000	6,121,000	5,386,000	6,336,000	6,574,000	6,616,000	7,250,000
4	W. B. C.	9,800	9,166	6,916	9,100	9,600	10,100	11,250	12,600	10,433
	R. B. C.	6,176,000	6,081,000	6,194,000	6,253,000	6,377,000	7,530,000	6,856,000	6,481,000	7,244,000
Averages....	W. B. C.	8,450	8,499	7,041	10,450	8,716	8,850	9,025	10,800	9,483
	R. B. C.	6,224,000	5,808,500	5,989,000	6,187,000	5,886,500	6,933,000	6,715,000	6,548,500	7,247,000
Percentage relations	W. B. C.	100.00	100.57	83.31	123.66	103.14	104.73	106.80	127.69	112.22
	R. B. C.	100.00	93.35	96.25	99.44	94.60	111.39	107.89	105.21	116.43

Because the blood picture did not show progressive change during the last two weeks of the experiment, we concluded that maximum effect had been obtained. Accordingly the animals were killed and autopsies performed at this time, the method of procedure, practically that described by Livingston (3), being briefly as follows: Each was killed by illuminating gas and weighed before and after expressing the urine. They were then freely bled by being suspended head downward and the abdomen compressed after cutting both carotid arteries and jugular veins. The alimentary tract from the cardia to the anus was excised, weighed and reweighed after its contents had been expressed. We now had the reduced body weight, i.e., the weight of the animal minus the urine and contents of the gastro-intestinal canal.

In general the gross appearance of the tissues was normal in all four animals. Careful inspection showed absolutely no evidence of any infection having existed. The thyroids, spleen and liver were removed from each rabbit and weighed. These weights expressed in milligrams or grams per kilogram of reduced body weight, together with the data from which the reduced body weight was computed, are given in table 3.

TABLE 3

ANIMAL	NUMBER	WEIGHT WHEN KILLED	WEIGHT MINUS URINE	WEIGHT OF GASTRO-INTESTINAL TRACT AND CONTENTS	WEIGHT OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	REDUCED BODY WEIGHT R. B. W.	WEIGHT OF THYROIDS IN MILLIGRAMS PER KILO OF R. B. W.	WEIGHT OF SPLEEN IN MILLIGRAMS PER KILO OF R. B. W.	WEIGHT OF LIVER IN GRAMS PER KILO OF R. B. W.
Secretin......	1	1890.0	1883.0	356	207.0	1734.0	138.4	991.9	44.994
	2	2249.0	2242.0	322	179.0	2099.0	94.8	819.4	40.752
	Averages	2069.5	2062.5	339	193.0	1916.5	116.6	905.65	42.873
Control......	3	2649.0	2649.0	451	304.0	2502.0	86.3	479.6	46.163
	4	1867.0	1844.0	299	179.0	1724.0	64.3	330.6	32.215
	Averages	2258.0	2246.5	375	241.5	2113.0	75.3	405.1	39.189

The thyroids in the secretin rabbits were 54.84 per cent heavier than in the controls, a greater difference than one would expect from individual variations as reported by Livingston (4). Also rabbits 1 and 2 showed slight enlargement of the liver, 9.37 per cent, as compared with the others; and the spleen was more than twice as large as in rabbits 3 and 4, 123.56 per cent.

There was no obvious enlargement of the lymph glands in the secretin rabbits though the cervical and abdominal chains were readily found. Rabbit 3, one of the controls, showed excessive subcutaneous fat, rendering the isolation of lymph nodes in this animal unsatisfactory. Lymph glands from each of the other three were preserved for histological examination.

Finally the head of the tibia in each animal was split open and the cylinder of red marrow carefully removed. In the controls this was of a light pink color, quite soft and very friable. In the others it was considerably darker in color, much more firm and decidedly less friable. These cylinders of marrow were also preserved for histological study. In addition smears of the marrow were made, several from each specimen and as uniform in thickness as possible. These were stained with Wright s stain by the same method employed for blood smears. The difference in the consistency of the marrow from the two pairs of rabbits was very noticeable in the making of the smears. When they were examined microscopically striking differences were observed strongly suggestive of increased activity on the part of the bone marrow of the secretin rabbits.

On the slides from the controls the cells were not numerous being mostly myelocytes with occasional large mononuclear lymphocytes and polymorphonuclear leucocytes. Nucleated red corpuscles were comparatively infrequent. In many fields they were not present and very rarely was more than one seen in a single field. The nuclei of these erythroblasts were deeply stained and quite uniform in appearance. On the other hand, in the smears from the secretin rabbits cells of all types were much more numerous, the most pronounced difference being seen in the nucleated red cells. One or more of these was found in every field and not infrequently as many as six or eight and often more were present in a single field. Furthermore, in most of these the nuclear network was plainly distinguishable and many of these cells were observed which were apparently undergoing cell division presenting various stages of mitosis. Several erythroblasts were also encountered in which the nucleus appeared to be undergoing extrusion. A number of myelocytes were also observed in the process of division. All types of leucocytes were likewise more numerous in these smears.

The absolute increase in the number of cells in one set of slides as contrasted with the other can hardly be attributed solely to unavoidable differences in thickness as the same variation was uniformly shown by all of them. The repeatedly observed evidence of cell division certainly would seem to indicate increased activity.

The cylinders of red marrow and the lymph glands were fixed in Müller's-formalin solution and embedded in celloidin. Sections were cut of a uniform thickness of eight microns and stained with Ehrlich's haematoxylin and alcoholic eosin.

The appearance of the sections of bone marrow from the controls corresponded closely with the usual depiction of normal red marrow, consisting of a rather loose network of cells with large spaces probably previously filled with fat. In the other sections the supporting reticulum could with some difficulty be made out but the spaces were closely packed with cells. These were chiefly myelocytes and erythroblasts with the former predominating. Only rarely small vacuoles were seen, fat cells probably. The myelocytes and erythroblasts, as in the smears, presented evidence of cell division. The absolute number of non-nucleated red corpuscles was greater in the secretin sections than in the controls. There can therefore be no doubt that the bone marrow was much more active in the rabbits which had been given secretin than in those to whom saline had been administered.

In the case of the lymph glands the evidence of increased activity was less striking. While the glands grossly were not obviously enlarged they were very readily found in the secretin rabbits even though no. 2 was quite as fat as no. 3 of the controls in which we were unable to isolate any glands satisfactorily for sectioning. The lymph gland sections showed the cells more closely packed in the glands from the secretin rabbits than in those from the controls. In the former the cells almost overlapped in some cases, whereas in the latter they were surrounded by free spaces at least as wide as the cells and usually wider.

The number of white corpuscles in the circulating blood of the secretin rabbits would seem to be directly proportional to their increased production, but the evidence of increased production of red corpuscles far surpasses the increase in the erythrocyte count. For this reason the question naturally presents itself: If such greatly increased activity of the bone marrow is produced by secretin, why is there not a greater and more persistent increase in the number of erythrocytes in the circulating blood? A clue to the answer to this question would seem to be afforded by the enlargement of the liver and spleen. According to Robertson and Rous (5) overactivity on the part of the bone marrow results in the production of immature erythrocytes whose resistance to disintegration in the blood stream is below normal. The remains of these corpuscles which have gone to pieces throughout the circulation are removed from the blood chiefly by the spleen but partly also by the liver. The accumulation of this debris, according to the same authors, is the chief cause of the enlarged spleen in anaemias. Possibly we have a similar condition brought about by the repeated administration of secretin, which is obviously producing overstimulation and therefore conceivably causing the production of less perfect corpuscles which undergo disintegration in the blood stream and are removed by the spleen and liver.

Another explanation of the apparent discrepancy between the production of the red corpuscles and the number in circulation is also to be found in the activity of the liver. It has been repeatedly demonstrated that secretin stimulates the secretion of bile (6), (7). Possibly this increased production of bile requires and brings about an increased destruction of the red corpuscles which is only a little more than offset by their increased production. Here again the enlargement of the spleen would have to be explained by accumulation in it of fragmented corpuscles. A direct relation between the disintegration of the red corpuscles with the liberation of haemoglobin and the secretion of bile pigment is claimed by Eppinger and Charnas (8), Wilbur and Addis (9) but denied in the more recent work of Whipple and Hooper (10).

Further evidence of the production of new corpuscles in response to secretin can be adduced from the study of the blood smears. The material for this study was obtained coincidentally with the making of the leucocyte counts in the experiments recorded in a previous paper (2) and, as previously mentioned, from the animals used in the preparation of the present report. The blood smears were stained according to the method recommended by Russell (11), viz., Wright's stain, 2 minutes; water, 5 minutes; dilute Manson's stain, 40 seconds; washed and dried. In every case at least two hundred cells were counted for deriving the percentages and the usual number counted was three hundred. Ehrlich's classification of the white corpuscles has been followed simply because it is so widely known.

We have made altogether sixteen determinations of the differential leucocyte count in apparently normal rabbits as they came to us before they were subjected to any experimental procedure. An average of the sixteen determinations gives us the following figures: Total count, 10,372 white corpuscles per cubic millimeter of blood; small mononuclear lymphocytes, 7.5 per cent; large mononuclear lymphocytes, 13.3 per cent; transitional leucocytes, 5.4 per cent; polymorphonuclear neutrophilic leucocytes, 69.7 per cent; polymorphonuclear eosinophilic leucocytes, 3.6 per cent; polymorphonuclear basophilic leucocytes, 0.5 per cent (table 4).

Differential leucocyte counts were also made from smears obtained at the time of maximum count in ten experiments in each of which the rabbit had been given subcutaneously a single dose of 1 cc. of secretin solution (10 mgm. of the dried acid extract) per kilogram of body weight. The details of these experiments have been recorded previously (2). Averaging these ten counts we get the following figures: Total count, 15,113 white corpuscles per cubic millimeter, which was an average increase of 44.2 per cent as compared with the initial counts in the same ten experiments; small mononuclears, 14.01 per cent; large mononuclears, 14.28 per cent; transitionals, 4.3 per cent; polymorphonuclear neutrophiles, 64.55 per cent; polymorphonuclear eosinophiles, 2.42 per cent; polymorphonuclear basophiles, 0.44 per cent (table 5).

TABLE 4

Differential leucocyte counts in normal rabbits

NUMBER	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
1	9,300	14.2	11.0	6.8	63.5	4.0	0.5
2	10,400	10.3	15.0	6.0	66.0	2.0	0.7
3	7,100	6.0	16.0	6.0	68.0	3.0	1.0
4	9,800	9.0	12.0	5.0	69.7	4.0	0.3
5 (1)*	4,800	7.0	13.3	4.5	67.6	7.3	0.3
6 (2)	10,400	7.0	9.0	5.0	74.0	4.7	0.3
7 (3)	9,600	5.0	15.0	6.0	69.0	4.5	0.5
8 (4)	6,200	2.7	10.7	5.6	77.0	3.5	0.5
9 (5)	11,600	10.3	11.3	3.0	72.0	3.0	0.4
10 (6)	20,000	8.0	13.5	4.0	70.0	4.0	0.5
11 (7)	15,600	10.0	13.5	6.0	67.0	3.0	0.5
12 (8)	12,654	4.0	17.0	6.0	70.5	2.0	0.5
13 (9)	10,900	7.0	18.0	6.0	65.5	3.0	0.5
14 (10)	7,400	8.5	10.5	5.5	73.0	2.0	0.5
15 (15)	11,000	5.0	15.0	6.0	70.0	3.5	0.5
16 (16)	9,200	6.0	12.0	5.0	72.4	4.1	0.5
Averages.......	10,372	7.5	13.3	5.4	69.7	3.6	0.5

* In this and succeeding tables the bracketed figures are the experiment numbers of previous report (2).

TABLE 5

Differential leucocyte counts at time of maximum effect following single dose of secretin

NUMBER	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
5 (1)	7,800	16.0	6.0	3.0	71.0	3.0	1.0
6 (2)	13,600	17.4	9.0	4.0	67.0	2.3	0.3
7 (3)	11,250	21.0	6.0	3.0	67.0	2.5	0.5
8 (4)	14,200	10.0	11.0	4.5	71.0	3.0	0.5
9 (5)	16,600	21.0	13.0	3.0	60.0	2.7	0.3
10 (6)	34,800	12.0	21.0	5.0	60.0	2.0	0.0
11 (7)	11,786	9.3	19.3	6.0	62.0	2.7	0.3
12 (8)	15,800	8.0	20.0	5.0	64.5	2.0	0.5
13 (9)	13,100	13.0	21.0	5.0	58.5	2.0	0.5
14 (10)	12,200	12.0	16.5	4.5	64.5	2.0	0.5
Averages.......	15,113	14.01	14.28	4.3	64.55	2.42	0.44

Following the administration of secretin, therefore, there is an absolute increase in all varieties of the white corpuscles, a considerable relative increase in the number of the small mononuclear lymphocytes and a slight relative increase in the large mononuclear lymphocytes, with a relative diminution in the polymorphonuclear leucocytes.

Tables 6 and 7 give the total and differential leucocyte counts in two experiments, also previously recorded in full (2), in each of which 1 cc. of secretin solution (10 mgm. of the dried acid extract) per kilogram of body weight was injected subcutaneously at hourly intervals for three doses. Here again there is a relative increase in the mono-

TABLE 6

Experiment 15 (15)

TIME	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
Initial............	11,000	5.0	15.0	6.0	70.0	3.5	0.5
1st hour..........	13,300	10.0	17.5	4.0	65.5	2.5	0.5
2d hour...........	11,600	10.5	16.0	5.0	63.5	4.0	1.0
3d hour...........	11,400	15.0	11.0	3.0	66.0	4.0	1.0
5th hour..........	14,600	7.5	10.0	3.0	75.5	3.5	0.5
6th hour..........	11,800	3.0	7.5	3.5	82.0	3.5	0.5

TABLE 7

Experiment 16 (16)

TIME	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
Initial	9,200	6.0	12.0	5.0	72.5	4.0	0.5
1st hour	15,300	10.0	16.0	5.0	65.0	3.5	0.5
2d hour	13,200	7.7	17.0	5.3	65.7	4.0	0.3
3d hour	12,600	7.0	17.0	4.5	67.5	3.5	0.5
5th hour	16,200	3.5	12.5	3.0	76.5	4.0	0.5
6th hour	12,200	3.0	9.0	4.5	79.0	4.0	0.5

nuclear lymphocytes. Toward the end of the experiment the opposite condition prevails, viz., a relative increase in the polymorphonuclear leucocytes with a relative diminution in the lymphocytes, persisting even after the falling off of the total count. Moreover, we five times observed nucleated red corpuscles in the blood smears of this series, in each instance in a smear obtained after the administration of the third dose of secretin.

We have further recorded the total and differential counts in each of the four rabbits of the present series at irregular intervals throughout the course of the experiment. These figures are given in tables 8, 9, 10 and 11. In this case the secretin rabbits show a slight relative increase in the large mononuclear lymphocytes and also the transitionals with a relative decrease in the small mononuclear lymphocytes and with practically no change in the proportion of the polymorphonuclear leucocytes. For example, averaging the initial counts we get: Total count, 9,850; small mononuclears, 12.25 per cent; large mononuclears, 13.0 per cent; transitionals, 6.5 per cent; polymorphonuclear neutrophiles, 64.75 per cent; polymorphonuclear eosinophiles, 3.0 per cent; polymorphonuclear basophiles, 0.5 per cent; and averaging all the counts made after administration of secretin we get: Total count, 16,300; small mononuclears, 9.37 per cent; large mononuclears, 15.2 per cent; transitionals, 9.56 per cent; polymorphonuclear neutrophiles, 62.12 per cent; polymorphonuclear eosinophiles, 3.0 per cent; polymorphonuclear basophiles, 0.5 per cent. A comparison of the differential counts of the control rabbits fails to show similar variations in the relative proportions of the different types of white corpuscles.

TABLE 8

Differential leucocyte counts in secretin rabbit, no. 1

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	9,300	14.0	11.0	7.0	63.5	4.0	0.5
December 19, 1917....	19,600	14.5	9.5	10.5	63.0	2.0	0.5
December 31, 1917....	15,600	10.0	10.0	8.0	68.5	3.0	0.5
January 26, 1918....	18,000	7.0	17.5	10.0	62.0	3.0	0.5
February 2, 1918....	15,400	8.0	17.0	9.0	62.5	3.0	0.5

TABLE 9

Differential leucocyte counts in secretin rabbit, no. 2

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	10,400	10.5	15.0	6.0	66.0	2.0	0.5
December 19, 1917....	11,600	9.0	16.0	8.0	63.0	2.5	0.5
December 31, 1917....	20,800	10.0	18.0	10.0	57.0	4.0	0.5
January 26, 1918....	15,200	7.5	17.5	11.0	59.5	4.0	0.5
February 2, 1918....	14,200	9.0	16.0	10.0	61.5	3.0	0.5

TABLE 10

Differential leucocyte counts in control rabbit, no. 3

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	7,100	6.0	16.0	6.0	68.0	3.0	1.0
December 19, 1917....	9,200	6.0	17.0	6.0	67.0	3.7	0.3
December 31, 1917....	7,400	5.0	19.0	5.0	66.3	4.0	0.7
January 26, 1918....	10,800	7.0	14.0	5.0	70.5	3.0	0.5
February 2, 1918....	9,200	5.0	16.0	5.0	69.5	4.0	0.5

TABLE 11

Differential leucocyte counts in control rabbit, no. 4

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	9,800	9.0	12.0	5.0	69.7	4.0	0.3
December 19, 1917....	6,600	6.0	17.0	6.0	66.5	4.0	0.5
December 31, 1917....	8,200	10.5	15.0	6.0	65.0	3.0	0.5
January 26, 1918....	13,200	8.0	12.0	6.0	69.5	4.0	0.5
February 2, 1918....	10,400	8.0	16.0	5.0	66.5	4.0	0.5

Such alterations in the relative percentages of the different forms of leucocytes, as have been recorded in tables 4 to 11 inclusive, where there is an absolute increase in the total white corpuscle count, is presumptive evidence of the formation of new cells especially of the types relatively increased.

SUMMARY

Therefore, we conclude that the increase in the number of red and white corpuscles per cubic millimeter of circulating blood shown to take place in the rabbit after the administration of secretin is dependent upon increased production of new blood cells. This greater production is apparently due to stimulation of the bone marrow and lymph glands by secretin. The evidence on which this conclusion is based is: the autopsy findings, the changes in the smears of bone marrow, the histological alteration in both the bone marrow and the lymph glands, the variation in the relative proportions of the white corpuscles and the appearance of nucleated red corpuscles in the circulating blood.

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II. FURTHER STUDIES ON THE RESPONSE OF THE VASOMOTOR MECHANISM TO REFLEX AFFERENT NERVE STIMULATION

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It has recently been established that a slow rate of stimulation and a weak current favor the depressor responses, and a rapid rate of stimulation and a strong current favor the pressor responses of the vasomotor mechanism. In this connection we shall restrict ourselves to the discussion of the literature directly pertaining to the subject, and refer the reader to more general discussions in recent articles by Gruber (1) and by Hunt (2).

Gruber (1) demonstrated that the rate of the stimulation as well as the strength of the stimulus plays an important part in determining whether a reflex vasodilator response or reflex vasoconstrictor response will result upon stimulation of cut afferent nerves. He noted that slow rates of stimuli, 1 to 10 per second, were favorable for the reflex depressor response, whereas rapid rates, 15 to 20 per second, were favorable in the production of the reflex pressor response. These results have been confirmed by Hunt (2). He cites an experiment in which a "tetanizing" current (about 80 interruptions per second) produced a rise of 15 mm. of mercury. The same strength of current interrupted 6 times per second produced a fall of 14 mm. of mercury. In 1895 he explained the differences, fall and rise, in blood pressure obtained upon stimulating a cut afferent nerve on the hypothesis that there are present in the same nerve trunk two separate sets of afferent fibers—vasodilator and vasoconstrictor (3). To meet the differences in thresholds determined by Martin and Lacey (4), 7.5 for the depressor reflex and 280 Z units for the pressor reflex, Ranson and Billingsley (5) advanced the hypothesis that the same nerve fiber is connected with a vasodilator center and a vasoconstrictor center, and that the resultant effect of stimulation is determined by the difference in irritability of the two, i.e., a weak stimulus reaches or excites the dilator more readily than it does the constrictor center. According to Ranson and Billingsley, the "depressor path" is through a long fiber tract with few relays, whereas the "pressor path" is through a series of short relays.

Vincent and Cameron (6) claim that the fall in blood pressure produced upon the stimulation of cut afferent nerves is atypical and that the rise in pressure is the natural response. They attribute the fall in pressure largely to changes in respiration, i.e., "hyperrespiration," and believe it to be the result of (1) mechanical interference with the heart's action; (2) mechanical interference with the return of the blood to the heart. They report that the extent of the fall of pressure appears to be largely proportional to the violence of the respiratory activity. They say, "Sometimes when the respiration is very violent, a pure fall of blood pressure may take place (see fig. 5)," (pp. 54–64–77).

In their work they could not always satisfy themselves as to the different effects of weak and strong currents produced by sliding the secondary coil away from the primary coil. On several occasions, however, they were able to obtain a fall in pressure with a weak current and a rise with a strong current by changing the number of cells in the primary circuit. They say,

> This effect is interpreted by Reid Hunt as pointing to the existence of "depressor or reflex vasodilator fibres" in the sciatic and similar nerves. If this is the case, opening the thorax should not alter the qualitative result. So far we have been unable to notice the difference just recorded between weak and strong currents respectively when the thoracic cavity has been opened.

They failed to state the rate of interruption of the primary current. However, we presume from a study of their results that it was a tetanizing current. As has been said, this rate of interruption favors the production of the reflex vasoconstrictor rather than the reflex vasodilator response.

In the light of some of the previous work done by one of us (1) upon the vasomotor system, we could not reconcile ourselves to the idea that the fall in blood pressure could have been influenced in the least by the respiratory movements affecting the heart's activity. (See *1*, fig. 3.)

The present research was therefore undertaken to determine to what extent the fall in blood pressure could be due to respiratory interference and incidentally to determine the approximate thresholds for the depressor and pressor responses in dogs with a given rate of stimulation.

METHOD

Both dogs and cats were used in these experiments, in all cases with light ether anaesthesia. The skin was incised on the median line of the neck and the animal tracheotomized. The blood pressure was always registered from the left carotid artery. A signal magnet, which marked intervals of five seconds, was placed at the atmospheric pressure line of the manometer.

For reflex afferent stimulation, the saphenous, femoral, ulnar and peroneal nerves were used. Each nerve, as used, was isolated, cut and the central end fastened in a Sherrington shielded electrode (7). The nerve was kept warm and from being stretched when the secondary coil was moved by fastening the two flaps of skin snugly on either side of the electrode with paper clips.

The stimulating current was usually 0.1 ampere in the primary circuit, and the strength of the secondary current was determined in Z units according to the Martin method (8). In a few cases, 0.5 and 1 ampere in the primary current was used.

The rate of stimulation, with the exception of a few cases in which it was 23 per second, was 7 interruptions per second. No attempt was made to short circuit the make shocks as it was thought that this alternating effect would overcome any polarization which might take place in the nerve trunk at the point of stimulation. The method of interrupting the current was the same as that employed in a previous research by one of us (1).

The effect of central nerve stimulation was tested both before and after the opening of the thorax in the experiments on dogs. In the experiments upon cats it was thought unnecessary to stimulate the nerve before the thorax was open, on account of the uniformity of previous results.

In all the experiments the ribs were transected on the side of the thorax and the entire sternum and parts of the ribs attached to it removed. This completely exposed the heart and lungs. Artificial respiration was carried on by a motor interrupting an air blast. Care was taken to maintain as nearly as possible a body temperature of 38°C. This was successfully done by placing an electric lamp over the animal.

RESULTS

The threshold stimulus. The threshold stimulus for the depressor response varied in the seven readings on dogs with the thorax closed from 4.2 to 20 Z units, or an average of 8.3 Z units. The stimulus necessary to bring about the pressor response varied in the same animals from 220 to 2425, or an average of 628 Z units.

Readings were made on animals with the thorax opened. The threshold for the depressor response varied in these from 4.2 to 16.8, with an average of 7.3 Z units, and for the pressor response 59 to 3100, with an average of 844.8 Z units. (See table 1.)

TABLE 1

The approximate threshold stimuli for pressor and depressor responses in dogs. Rate of stimulation 7 times per second

THORAX CLOSED			THORAX OPENED		
Nerve	Approximate threshold fall in Z units	Approximate threshold rise in Z units	Nerve	Approximate threshold fall in Z units	Approximate threshold rise in Z units
Left saphenous	4.6	485	Left saphenous	4.2	295
Right saphenous	4.2	485	Femoral	4.2	220
Ulnar	20.3	485	Ulnar	16.8	220
Right saphenous	4.2	220	Peroneal	4.2	59
Right saphenous	4.2	485	Femoral	4.2	1100
Ulnar	16.8	220	Left saphenous	4.2	3100
Saphenous	4.2	2425	Peroneal	4.2	2425
			Saphenous	7.5	485
			Peroneal	16.8	59
			Saphenous	7.5	485
Average	8.3	628		7.3	844

The average for the depressor response in these experiments upon dogs is the same as that obtained by Martin and Lacey (4) in experiments on decerebrate cats but lower by 40 per cent than the results they obtained with ether or urethane anaesthesia on the same experimental animal. This difference is probably due to the difference in the depth of anaesthesia.

The average current strengths of 844 and 628 which we found necessary to produce a rise in blood pressure are much higher than that found by Martin and Lacey, 280 Z units.

Optimum strength of stimulus. With a rate of stimulation of 7 per second the maximal fall in blood pressure was obtained in 80 per cent of the experiments with a current of 16.8 Z units in the animals with the thorax closed or opened. The average fall in blood pressure with the thorax closed, tested upon 5 dogs and 7 nerves, was 15 mm. of mercury, or a fall of 9.5 per cent. The average fall in blood pressure tested upon eleven different nerves in five different animals with the thorax opened was 13.3 mm. of mercury, or a fall of 10.9 per cent. (See table 2.)

TABLE 2

The maximal fall in blood pressure in millimeters of mercury, and the fall in blood pressure in per cent, upon reflex afferent nerve stimulation in dogs. Rate of stimulation 7 per second

THORAX CLOSED				THORAX OPENED			
Nerve	Strength of current in Z units	Maximal fall in blood pressure in millimeters of Hg.	Fall in blood pressure in per cent	Nerve	Strength of current in Z units	Maximal fall in blood pressure in millimeters of Hg.	Fall in blood pressure in per cent
Saphenous	16.8	25	16.6	Saphenous	16.8	10.0	8.0
Saphenous	16.8	14	9.0	Peroneal	16.8	10.0	8.0
Ulnar	175.0	6	4.0	Ulnar	59	13.0	9.0
Saphenous	7.5	7	4.4	Ulnar	7.5	10.0	9.0
Saphenous	16.8	14	8.5	Femoral	16.8	21.0	16.0
Ulnar	7.5	8	5.0	Saphenous	16.8	20.0	14.5
Saphenous	16.8	31	19.0	Peroneal	16.8	18.0	13.0
				Saphenous	16.8	12.0	10.0
				Peroneal	16.8	8.0	10.0
				Saphenous	16.8	8.0	7.0
				Femoral	485	20.0	16.0
Average		15	9.5			13 3	10.9

That the depressor response is as readily elicited in dogs as we have previously shown it to be in cats can be seen in figure 1. The animal was under light ether anaesthesia. The rate of interruption of the stimulating current was 7 per second. The maximal fall was obtained at *3*, in which the strength of stimulus was 16.8 Z units. There occurred with this strength of stimulus applied to the saphenous nerve a drop in blood pressure from 150 to 126 mm. of mercury, a fall of

16 per cent. No noticeable change in respiration occurred during the experiment.

Figures 2 and 3 are records taken from the same animal as was figure 1, but with the thorax opened. The opening measured 4 by 6 inches. Upon stimulation of the femoral nerve in figure 2 the blood pressure decreased from 135 to 118 mm. of mercury, or a fall of 12.6 per cent at *2* with the same strength of stimulus as in figure 1, *3*.

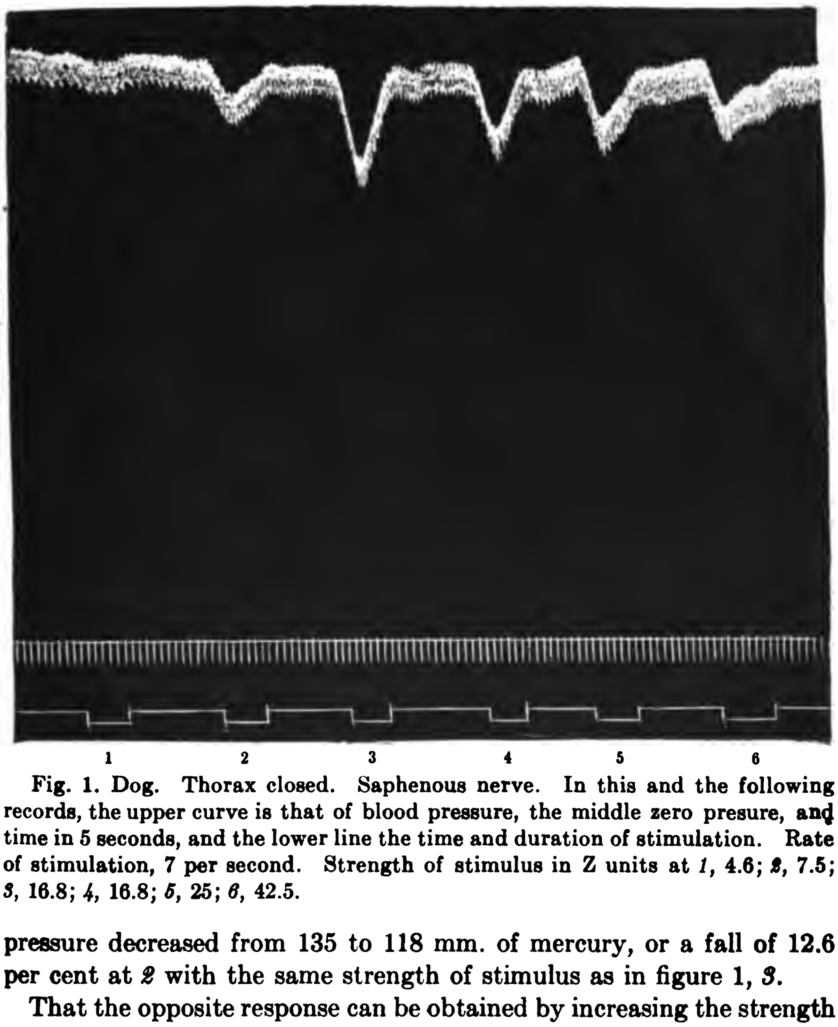

Fig. 1. Dog. Thorax closed. Saphenous nerve. In this and the following records, the upper curve is that of blood pressure, the middle zero presure, and time in 5 seconds, and the lower line the time and duration of stimulation. Rate of stimulation, 7 per second. Strength of stimulus in Z units at *1*, 4.6; *2*, 7.5; *3*, 16.8; *4*, 16.8; *5*, 25; *6*, 42.5.

That the opposite response can be obtained by increasing the strength of stimulus in animals with the thorax opened and with heart and lungs entirely exposed, is demonstrated in figure 3. At points *1*, *2* and *3* electrical excitation of the peroneal nerve produced pure falls in blood pressure. At *4* the reversal took place with a current strength of 59 Z units. In only one other case were we able to produce a rise in blood pressure with so weak a current. In all other nerves in which such a response could be obtained the strength of current necessary to bring about the reversal was 220 or more Z units. In a number of nerves we were unable to get the pressor response with any strength of current available. Figure 4 is presented to show that even very strong currents interrupted 7 times per second do not produce a rise in blood pressure. The femoral nerve was stimulated at *1* with a current of 485, at *2* with 2425 and at *3* with 4850 Z units. The blood pressure fell in each case and at *1*, 20 mm., or a decrease of 16.6 per cent.

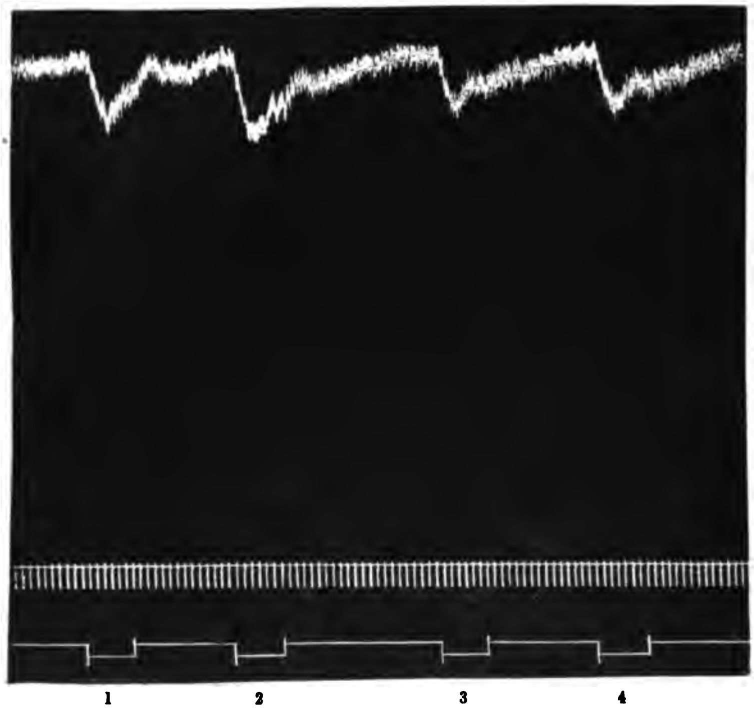

Fig. 2. Dog. Thorax opened. Femoral nerve. Strength of stimulus in Z units, *1*, 7.5; *2*, 16.8; *3*, 59.0; *4*, 175.5.

Similar curves were obtained from the cats used in these experiments. With a rate of stimulation of 7 per second we never failed to obtain a fall in blood pressure in animals with the chest closed or opened. The extent of the fall was modified by the depth of anaesthesia; the lighter the anaesthesia, the lower the threshold and the greater the extent of decrease.

The respiratory rôle. Our experiments do not support Vincent and Cameron's theory that the fall in blood pressure is brought about by movements of respiration which interfere with the heart's activity.

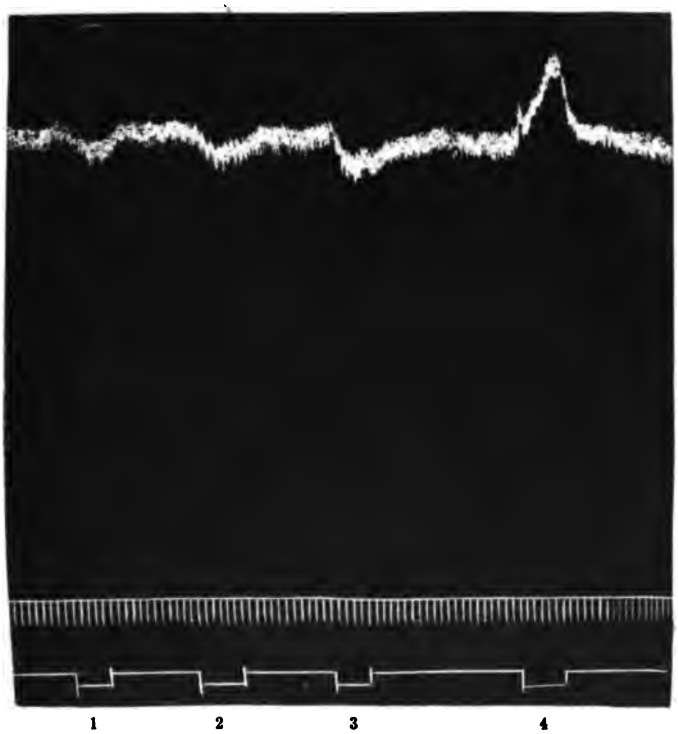

Fig. 3. Dog. Thorax opened. Peroneal nerve. Strength of stimulus in Z units, *1*, 4.2; *2*, 7.5; *3*, 16.8; *4*, 59.

They found that as the thorax was opened, the fall in blood pressure disappeared. They were unable to obtain a difference in the response of the vasomotor system to weak and strong stimuli when the thorax was opened. Only a rise was obtainable.

We found that not only was it possible to obtain a fall in blood pressure with the thorax opened, but that the threshold strength of stimulus was approximately the same in either case. (See table 1.)

The results in table 2 show that it is not only possible to obtain a fall in blood pressure independently of respiratory excitation and the resultant interference with the heart's activity, but that the extent of this fall is greater for animals with the thorax opened.

The differences observed between Vincent and Cameron's results and ours are in all probability due to differences in the rate of interruption of the stimulating current.

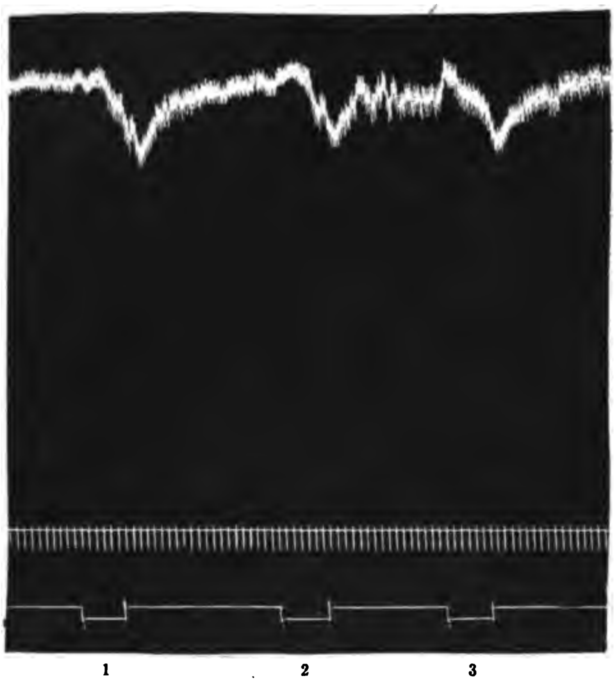

Fig. 4. Dog. Thorax opened. Femoral nerve. Strength of stimulus in Z units, *1*, 485; *2*, 2425; *3*, 4850.

SUMMARY

In our experiments, respiration affected in no manner whatever the production of the fall in blood pressure upon central afferent nerve stimulation. Opening the thorax had no effect upon the production of the depressor response from the vasomotor mechanism.

With a rate of 7 interruptions per second we found it extremely difficult to bring about pressor responses but depressor responses were readily elicited regardless of the condition of the thorax.

The average thresholds for the depressor response in dogs, 8.3 with thorax closed and 7.3 with thorax opened, are about the same as that found by Martin and Lacey upon decerebrate cats, 8.5 Z units.

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THE ACTION OF THE AUTONOMIC DRUGS ON THE SURVIVING STOMACH

A Study on the Innervation of the Stomach

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A study of the literature dealing with the innervation of the stomach and the effects of drugs upon it discloses that all such investigations were carried out on the whole organ, whether in situ or isolated. There does not seem to have been any consideration given to the possibility that the different parts of the viscus may have different innervations and that the effect of a given drug on one region of the stomach may not necessarily be the same as its effect on another portion of this organ.

That the innervation of the stomach may not be uniform at all points and in all animals, at least in as far as the sympathetic nerve supply of it is concerned, might be surmised from the fact that different observers have reported varying effects on the movements of this organ following the stimulation of the splanchnic nerves. Schiff (1), Morat (2), Dixon (3) and others have shown the splanchnics to supply motor fibers to the stomach, while Wertheimer (4) and Elliott (5) may be mentioned among those observers who found the stomach movements to be inhibited from the stimulation of the splanchnics. Openchowski (6) demonstrated both motor and inhibitory effects on the stomach of the dog and the rabbit following stimulation of the splanchnics, while May (7) on the other hand, stimulating the same nerves in the cat, dog, rabbit and monkey observed no appreciable effects on its movements.

As to the effects of drugs on the movements of the stomach, the work of Schutz (8) may be mentioned, who limited his observations to the action of a series of drugs on the whole isolated stomach of the dog. Later Glaessner (9) made similar observations on the whole excised stomach of the frog.

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[1] Some of the earlier experiments of this research were carried out in the pharmacological laboratory of the University of Michigan.

The action of suprarenal extract on the movements of the stomach undoubtedly received the greatest attention in the hands of various observers but no uniform results have been reported. Boruttau (10) working with rings of the frog's stomach found that suprarenal extract caused a relaxation of tonus. Elliott (5) observed similar effects from the extract on the cat's stomach, as did Langley (11) in the case of the stomach of the cat and the rabbit. Dixon (3) however found suprarenal extract to cause tonic contraction and augmented automatic movements of the frog's stomach.

METHOD AND SCOPE

The present work was carried out on strips of the surviving stomach suspended in oxygenated Tyrode's solution kept at constant body temperature. The movements of the strips were recorded on a slowly revolving kymograph by means of a light heart lever sufficiently weighted. The drugs were added in definite amounts to the Tyrode solution containing the suspended strip. Just as soon as the effects of a drug were noted the solution was drawn off and fresh Tyrode's solution, warmed to body temperature, was replaced. A period of at least five minutes and usually a longer interval of time elapsed before a second trial with the same or another drug was made.

The strips were generally taken from the following regions of the stomach: (1) antrum; (2) preantrum,—well defined in the rabbit's stomach, less so in that of the cat and dog; (3) body of the stomach, the portion extending from the preantrum to a point opposite the oesophageal insertion; (4) fundus, or the rounded cul-de-sac to the left of the oesophageal orifice.

In most cases longitudinal strips were used though at times strips corresponding to the circular as well as the óblique fibers of the stomach wall were employed. No material difference could be observed in the behavior of the longitudinal, circular or oblique strips of a given region toward the drugs used.

Both surfaces of the stomach, the anterior as well as the posterior, were examined. In a few instances strips from the lesser and greater curvatures were employed and several experiments were made with the pyloric and cardiac sphincters. At least two experiments were carried out for each region of either surface. As a rule fresh tissue was used but in a few experiments tissue that was kept in the cold for about twenty-four hours was used, which was found to respond perfectly well. Strips from the stomach of the guinea pig, rabbit, cat, dog and the human subject[2] were utilized in this work.

The observations were limited to the effects of those drugs that act on the autonomic nervous system in the hope that this might throw some light on the innervation of the several regions of the stomach. Epinephrin and nicotine were used as acting on the sympathetic structures, "receptive substance" and ganglia respectively, and pilocarpine and atropine, acting presumably on the parasympathetic endings and antagonistic to each other. Barium chloride was frequently employed to test the irritability of the tissue. Thus if a strip failed to respond to barium it was not considered suitable for this work, and it was discarded. It may be remarked however that often stomach strips failing to respond to barium chloride responded perfectly well to pilocarpine.

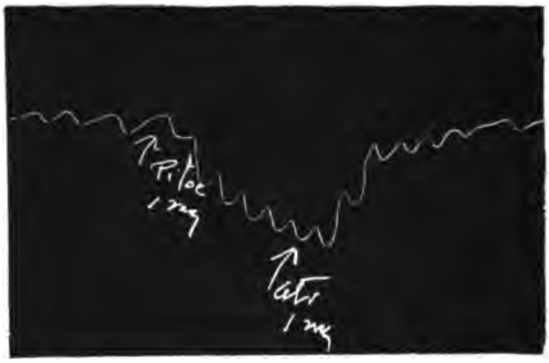

Fig. 1. Body of rabbit's stomach, anterior surface. Contraction and increased tonus from 1 mgm. pilocarpine hydrochloride. Relaxation from 0.1 mgm. atropine sulphate. (In this as in subsequent tracings contraction is indicated by downward movement of lever.)

RESULTS

Effects of pilocarpine and atropine. Pilocarpine and atropine produced a uniform effect upon all the regions of the stomach, and in all the species of animals studied. Pilocarpine uniformly produced a contraction in dilutions of from 1:10,000 to 1:100,000, while atropine antagonized the effects of pilocarpine in solutions one-tenth as strong, and produced a relaxation of the tissues. One typical illustration of the effects of pilocarpine and atropine is shown in figure 1. On adding 1 mgm. of pilocarpine hydrochloride to about 100 cc. Tyrode's solution in which a strip from the anterior surface of the body of the rabbit's stomach was suspended a marked contraction resulted. This was promptly counteracted, and relaxation occurred, upon the addition of 0.1 mgm. atropine sulphate. Similar effects were noted from these drugs on the pyloric and cardiac sphincters.

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[2] Owing to the kindness of the medical staff of the University Hospital it was possible for me to secure the stomach of a patient who died about three hours previously of ruptured aneurysm.

Effects of nicotine. A solution of the hydrochloride of nicotine was used. The effects of this drug were noted in dilutions of from 1: 10,000 to 1: 1,000,000 upon all regions of the stomach, including the sphincters, of all the animals studied. A tonic contraction such as is shown in figure 2, produced by 3 mgm. nicotine hydrochloride added to about 100 cc. Tyrode's solution in which a strip from the anterior surface of the human antrum was suspended, is the rule.[3] The contraction usually appeared promptly and soon passed off, so that the strip returned to its original condition, or it passed into a state of relaxation. The shortness in duration of the nicotine contraction in the above mentioned concentrations does not seem to be due to a paralysis of any local nerve mechanism for the addition of another dose of the drug to the same solution often produced a similar contraction, and repeated contractions were obtained from successive doses of the

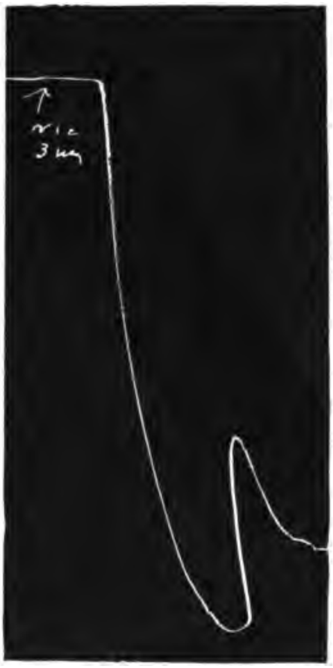

Fig. 2. Human stomach. Antrum, anterior surface. Shows tonic contraction from 3 mgm. nicotine hydrochloride.

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[3] In a number of experiments no effect could be obtained from nicotine, even though the strips contracted spontaneously and responded well to the other drugs employed.

drug when added to fresh Tyrode's solution at short intervals of time.

Occasionally the contraction produced by nicotine was preceded by a slight relaxation. This was noted in a few instances in the case of the antrum, body and fundus of the rabbit's stomach. The cardiac sphincter of the cat's stomach relaxed from nicotine, while the fundus relaxed in one experiment and contracted in two.

TABLE 1

Effect of epinephrin on the different regions of the stomach

STRIP	GUINEA PIG	RABBIT	CAT	DOG	HUMAN
Pyloric sphincter		Contraction	Contraction		Contraction
Antrum		Relaxation	Relaxation		Relaxation
Preantrum		Relaxation	Relaxation	Relaxation	
Body	Anterior surface and greater curvature relaxation; posterior surface and lesser curvature contraction	Contraction*	Relaxation	Contraction	Relaxation
Fundus		Contraction	Relaxation	Contraction	
Cardiac sphincter			Contraction	Contraction	

* Deviations from this are discussed in the text.

Effects of epinephrin. The hydrochloride of epinephrin[4] was used in dilutions varying from 1:100,000 to 1:10,000,000. The results of this drug are best presented in table form, from which it will appear that the response of the various strips to it is not the same for all regions of the stomach, nor do the strips of the same region of different animals respond alike.[5]

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[4] Made from adrenalin of Parke, Davis and Company.

[5] It may be recalled that small doses of epinephrin may produce the opposite effect of a larger dose. This was constantly borne in mind and for each result in the accompanying table several trials were made with varying doses.

The body of the stomach of the guinea pig, the only portion of this animal's stomach definitely worked out, presented a different reaction to epinephrin for its two surfaces and curvatures. Thus while the anterior surface and greater curvature relaxed, the posterior surface and lesser curvature strongly contracted.

In the other animals in which a complete record was obtained for all the regions of the stomach, considerable differences also prevailed. All the regions of the cat's stomach, with the exception of the sphincters, relax from epinephrin. The stomach of the rabbit, however, as well as that of the dog contracts in the main from epinephrin, although some regions relax. Thus, the antrum and the preantrum are the only parts of the rabbit's stomach that definitely and constantly relax. In one experiment the posterior surface of the body of the rabbit's stomach was noted to slightly relax before contracting (fig. 3). The region of the dog's stomach that uniformly relaxed from epinephrin is the preantrum. Strips from the antrum repeatedly failed to respond in any manner even though they were beating spontaneously and responded to barium chloride, pilocarpine, atropine and nicotine with great avidity. The body and the fundus of the dog's stomach were found to uniformly contract.

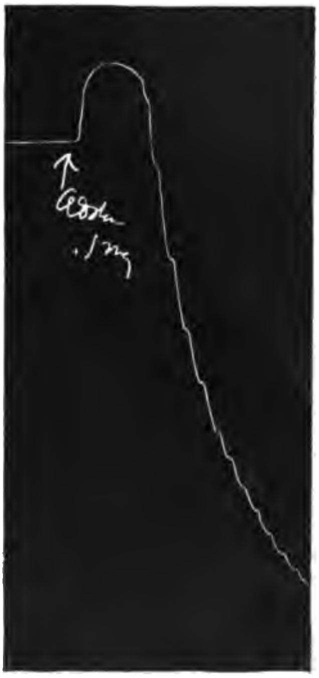

Fig. 3. Body of rabbit's stomach, posterior surface. Strong contraction preceded by slight relaxation, produced by 0.1 mgm. epinephrin hydrochloride.

That the reaction of the larger part of the rabbit's and the dog's stomach to epinephrin by a contraction is not merely a peculiarity of surviving tissue but that the same reaction prevails in the living animal was verified by two experiments on the rabbit and one on the dog in which the movements of the stomach in situ and the reaction to epinephrin were observed.

The animals were anesthetized, submerged in a bath of normal saline at 38°C., the stomach exposed by a median incision of the abdomen and the Cushny myocardiograph tied into the anterior surface of the body of the stomach to record the movements of a strip of about 4 cm.

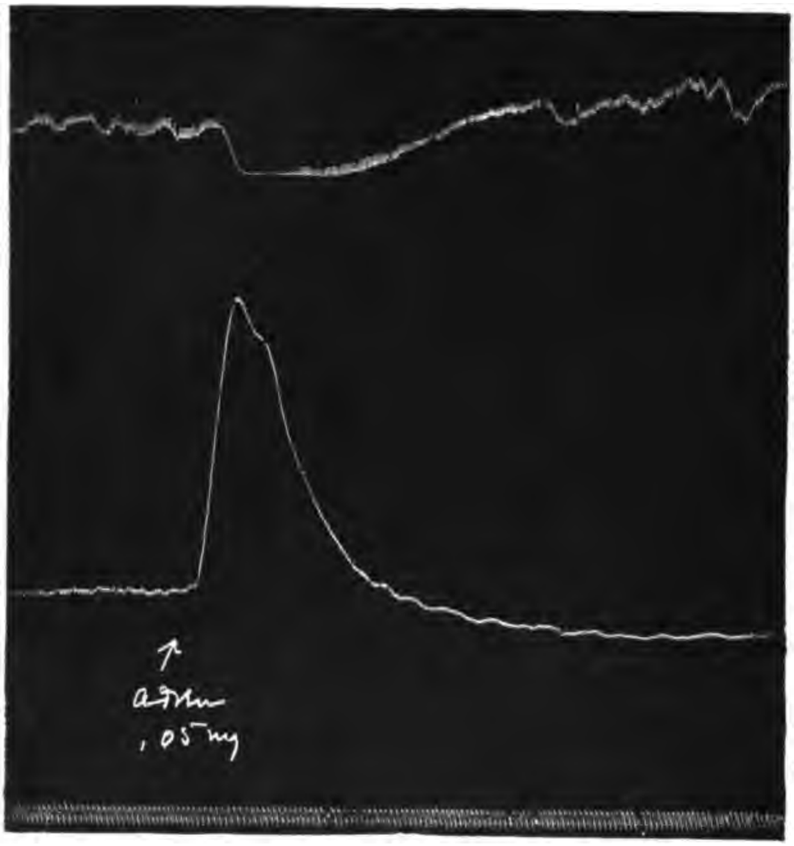

Fig. 4. Effect of epinephrin on the movements of the body of the rabbit's stomach in situ. Upper tracing, movements of stomach; middle tracing, blood pressure; lower tracing, time in seconds. Note the contraction of this portion of the stomach following the rise in blood pressure.

thereof. The blood pressure was recorded at the same time.[6] Following the injection of a small dose of epinephrin into the jugular vein there was the usual rise in blood pressure, and a well marked contraction of the portion of the stomach recorded, which lasted about the same length of time as the rise in blood pressure (fig. 4).

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[6] In the dog the vagi were divided and artificial respiration administered.

The effect of epinephrin on strips of the human stomach was that of relaxation in as far as it was possible to ascertain by the available material. Both surfaces of the antrum and the body of the human stomach were found to relax from this drug.

The effects of epinephrin on the sphincters were obtained with difficulty. The excised muscle of the sphincter is strongly contracted and thus responds slightly if at all to augmentory drugs. The pyloric sphincter of the stomach of the cat, rabbit and the human subject was found to contract slightly in a few instances while in most cases no reaction could be obtained. The cardiac sphincter of the cat's stomach contracted well from epinephrin and that of the dog's stomach but slightly. The cardiac sphincter of the rabbit's stomach failed to respond to this drug. It would seem that when a reaction to epinephrin is obtainable with the sphincters, it is a contraction, which is in agreement with the observations of Elliott on the effects of epinephrin on the pyloric sphincter of the cat (5). Langley (11) however has shown that suprarenal extract (1 cc. "tabloid 1 in 6") produced a relaxation of the cardiac sphincter of the rabbit. I am unable to corroborate this observation by the method I have used, and I can not account for the disparity of results.

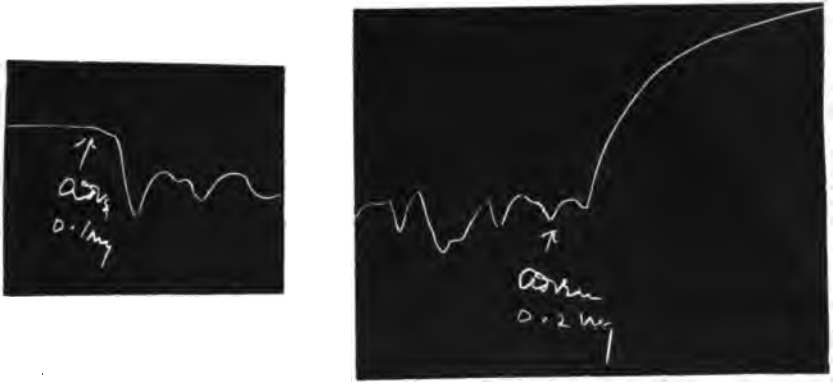
DISCUSSION

The interpretation of the results obtained with pilocarpine and atropine upon the stomach is rather dubious. The action of these drugs, it is generally held, is upon the parasympathetic nerve endings and it was hoped that the reaction of strips from different regions of the stomach to them might show the distribution of the vagus nerve to this organ. The uniform response of the various strips to pilocarpine by a contraction, and to atropine by a relaxation cannot be taken however as evidence that all the regions of the stomach are supplied by motor fibers from the vagus, since it has been shown by Openchowski (6), Langley (12), May (7), Elliott (5) and others that stimulation of the vagus relaxes at least some parts of the stomach, especially the region of the cardia. It must be remarked however that the relaxation of part or all of the stomach from vagus stimulation as reported in the literature is a primary effect and that the secondary effect, it is generally agreed, is a powerful contraction of the whole organ. It may well be that the primary relaxing effect upon some parts of the stomach resulting from stimulation of the vagus cannot be demonstrated on excised tissue. This is made more probable by the observation of Langley (12) that frequently the primary as well as the secondary effect from vagus stimulation is a contraction, and that in the exposed stomach the vagus inhibitory effects are less marked and often inconstant. The other alternative is that the contraction produced on all the parts of the stomach by pilocarpine is due to some mechanism other than through the vagus.

No very clear explanation can be set forth for the effects of nicotine upon the different regions of the stomach. It was found, as pointed out earlier, that as a rule, whenever effective, nicotine produces a contraction. In about one-third of the number of experiments, however, (about twenty out of fifty-five), the tissues although beating spontaneously and reacting well to the other drugs, failed to react to nicotine. Is this motor effect of nicotine when elicited to be ascribed to its action on ganglia, and is a positive effect on a given strip to mean the presence of ganglia, while a negative result is to indicate absence of ganglia in that particular strip? Such an explanation is plausible although it can not be stated with any degree of definiteness. While there are ganglionated structures scattered all through the walls of the stomach (plexuses of Auerbach and Meissner), special groups have been shown to occur in greatest abundance in the pyloric and cardiac regions (Openchowski (6), Keith (13)). An analysis of my experiments does not show strips from these regions to respond to nicotine more frequently than strips from other regions. Indeed, the greatest number of failures to respond to this drug occurred among the strips taken from the antrum, while those from the preantrum responded in all cases. Strips from the fundus (region of the cardia) compare with those taken from the body of the stomach as regards their reaction to nicotine.

The significance of the reaction of the different regions of the stomach to epinephrin is clear. Lewandowski (14), Langley (11), Elliott (5) and others have shown that the effect of epinephrin on a tissue corresponds to the stimulation of the sympathetic innervation thereof and that the action of the drug is augmentory or inhibitory depending upon as to whether the corresponding sympathetic nerve supply is motor or inhibitory. The different reactions of the various strips to epinephrin clearly show that the sympathetic innervation of the stomach is not the same in all animals and is different for different regions of the stomach in the same animal. It appears that of the usual laboratory animals the cat alone has an inhibitory sympathetic nerve supply of the whole stomach, with the exception of the sphincters. This confirms the observations of Elliott (5) who found by recording the volume changes of the cat's stomach a complete relaxation of the whole organ upon the administration of epinephrin as well as upon the stimulation of the splanchnics.

The sympathetic innervation of the rabbit's and dog's stomach on the other hand, is augmentory in the main, except that of the antrum and preantrum of the former and the preantrum of the latter animal, since only the antrum and preantrum of the rabbit and the preantrum of the dog relax from epinephrin, while the other regions contract.



a b

Fig. 5. Body of dog's stomach. Posterior surface. Effect of epinephrin before and after ergotoxine. *a*, 0.1 mgm. epinephrin causes a contraction. *b*, 0.2 mgm. epinephrin, after 2 mgm. ergotoxine, produces a relaxation.

That the parts of the dog's stomach (and probably of the rabbit's as well) contracting from epinephrin are not devoid of sympathetic inhibitory fibers, was shown by the application to the stomach of Dale's epinephrin vasomotor reversal produced by ergotoxine (15). A strip from the posterior surface of the body of the dog's stomach was suspended in the usual manner in Tyrode's solution and treated with a small dose of epinephrin. The usual contraction followed. The solution was withdrawn, fresh Tyrode's solution replaced and about 2 mgm. ergotoxine were added to this. After a few minutes the reaction of the strip to epinephrin was again tested. Instead of the usual contraction a very marked relaxation occurred (fig. 5). A similar reversal was obtained in another experiment on the fundus of the dog's stomach This proves in another way the earlier observation of Openchowski (6) that the sympathetic nerve supply of the dog's stomach is both motor and inhibitory.

The sympathetic innervation of the human stomach, as can be inferred from the reaction of the strips to epinephrin, is inhibitory, except of course the sphincters.

It seems contrary to expectations that the innervation of the dog's stomach is more like that of the rabbit's than that of the cat's. Besides the dog being a carnivorous animal like the cat and unlike the rabbit, it is a matter of common observation that anatomically the dog's stomach conforms more closely to that of the cat than to that of the rabbit. Physiologically too, in the matter of absorption, and the ease with which the dog can be induced to vomit would put his stomach nearer that of the human and the cat than that of the rabbit. It is perhaps justifiable to conclude that the sympathetic innervation of the stomach has nothing to do with the process of vomiting since the innervation of the stomach of the dog that can be induced to vomit very readily is very much like the sympathetic innervation of the stomach of the rabbit, that is entirely incapable of vomiting.

SUMMARY AND CONCLUSIONS

1. Pilocarpine causes a contraction of all regions of the surviving stomach of the guinea pig, rabbit, cat, dog and the human subject. Atropine antagonizes the action of pilocarpine and produces a relaxation.

2. Nicotine likewise produces a contraction of all parts of the stomach of the animals enumerated, except some parts of the cat's stomach (fundus, cardiac sphincter) which may relax, and some parts of the rabbit's stomach (antrum and body) which may slightly relax before contracting.

3. The reaction of the different parts of the stomach to epinephrin may be that of relaxation (cat and human), or relaxation of some parts and contraction of others (guinea pig, rabbit, dog). The reaction of the sphincters to epinephrin is always that of contraction.

4. Those regions of the dog's stomach that contract from epinephrin can be made to relax therefrom after ergotoxine, showing that there is

an inhibitory sympathetic innervation to those parts as well as an augmentory, although the latter predominates normally.

5. It is concluded from this that the sympathetic innervation of the stomach is inhibitory in some animals (cat and the human), while in other animals (guinea pig, rabbit and dog) it is inhibitory for certain regions and predominantly augmentory for others.

The sympathetic innervation of the sphincters of the stomach appears to be augmentory.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

I. NORMAL TEMPERATURE VARIATIONS AND THE TEMPERATURE EFFECTS OF OPERATIVE PROCEDURES

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INTRODUCTION

This paper is the first of a series of experimental studies on the various physiological and anatomical factors which tend to bring about the relatively constant temperature found in birds and mammals.

It is commonly agreed that two fundamental processes are involved in the regulation of temperature: heat production and heat dissipation. Anything which increases destructive metabolism increases heat production and tends to bring about a corresponding rise in body temperature. If, on the other hand, the heat production remains constant, the temperature varies according to the amount of heat given off or retained. It is recognized that loss of heat occurs through radiation and conduction and through evaporation of water. The loss through radiation and conduction is affected by vasomotor changes; that by evaporation is affected by vasomotor changes and by the rate of activity of the sweat glands and by the rate and depth of the breathing movements. Body temperature must be thought of, therefore, as a balance between heat production and heat dissipation, the balance remaining fairly constant in all warm-blooded animals.

How is the balance maintained? Many workers have assumed the existence in the brain of a convenient, hypothetical "heat center," which acts as a thermo-regulator, automatically altering the heat production or dissipation so as to maintain the balance in ordinary conditions. If the center is stimulated, a higher temperature results; if it is depressed or destroyed, the temperature falls. These writers have differed among themselves as to the location, number and exact function of the centers assumed.

In contrast to these views, others think of the heat regulation as analogous to the regulation of blood pressure. The control of blood pressure is never ascribed to a specific center but is regarded rather as the mean result of various physico-chemical factors. There is an even greater constancy in the acidity and calcium content of the blood, which is accounted for by physical and chemical equilibria without the intervention of special "centers." A similar balance between heat production and heat loss might well result in a constant body temperature. It does not seem to have been thought necessary to assume "centers" for the control of the osmotic pressure of the blood nor of the number of red corpuscles to the cubic millimeter.

As Henderson (1) says:

> Further research reveals similar equilibria concerning carbon, sulphur, phosphorus and other elements . . . . water, salt, sodium bicarbonates, glucose and the like. It is perceived that the equilibria of *temperature*, of volume, of alkalinity, which involve physico-chemical states are truly analogous phenomena.

Since the available experimental evidence for a specific temperature regulatory mechanism, though profuse, is conflicting and often unconvincing, it seemed worth while to investigate further the factors concerned. Before beginning the experiments necessary for this purpose, it was imperative to determine, first, the range of normal temperature of the animals used; and second, the variable conditions which might exert an effect on their temperature and thus prove a source of error in the experimental results. With this object in view the observations recorded below were obtained. Rabbits were employed as the experimental animals.

The author gratefully acknowledges the guidance of Prof. S. S. Maxwell in this investigation.

I. THE NORMAL TEMPERATURE OF THE RABBIT

It is impossible to establish a temperature norm for the rabbit because of its extreme variability in this animal. The range however, has rather definite limits. Pembrey (2) gives extremes of 37° and 40.8°C.; Simpson and Galbraith (3) 39° and 40°C.; Krehl (4) 38.3° and 39.9°C.; Freund (5) 38.6° and 39.6°C.; Davidson and Friedman (6) 38.5° and 40°C.; Bock (7) 38.6° and 40.9°C.; Burnett (8) 38.6° to 40°C.; Hale

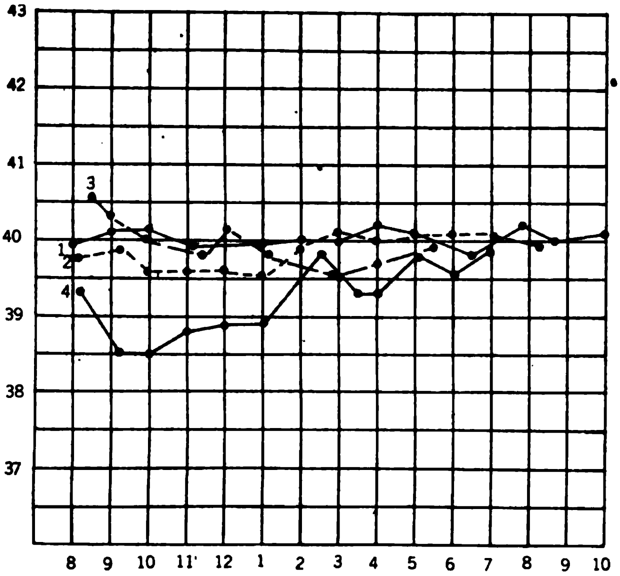

Chart 1. Daily temperature variations in rabbit 7. Ordinates, degrees Centigrade; abscissae time in hours. *1*, November 17; *2*, November 13; *3*, November 7; *4*, November 24.

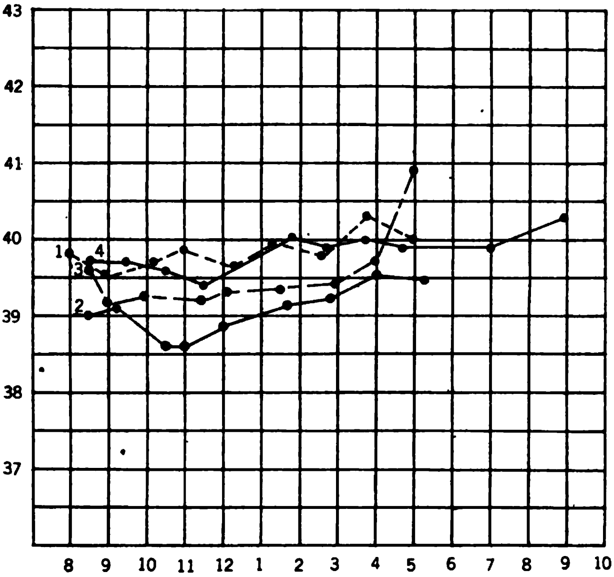

Chart 2. Daily temperature variations in rabbit 10. Ordinates, degrees Centigrade; abscissae time in hours. *1*, December 5; *2*, December 6; *3*, December 7; *4*, December 14.

White (9) 37.3° and 39.9°C. Frothingham and Minot (10) decide from a series of two readings daily that although these and other observers find variations from 2° to 4°C. a constant range of 2°C. would be utilizable in drawing valid conclusions in experimental work. The data given in this article were obtained from observations on 22 rabbits. The observations extended over a period of eighty-six days and included 774 readings.

Method. The rabbits were kept in large boxes in the experimental room and were never moved except to make the observations. They were then handled in such a way as to cause as little excitement as possible, struggling seldom occurring. They were fed on barley and hay daily about 6 p.m. The temperature was taken per rectum by standardized clinical thermometers inserted to a depth of two or more inches and left in for two minutes. The temperatures were recorded hourly from 8 a.m. to 6 p.m. and in many cases to 9 or 10 p.m. The observations of eleven rabbits were made on from two to thirteen successive days, the animals being kept as nearly normal as possible in the meantime. In the remaining cases the readings were taken during a single day for each rabbit.

Charts 1 and 2 are specimen curves of the daily temperature variations of two rabbits. In some cases the range was much greater, one varying from 38.4° to 41.2°C. in the course of one day. The extreme of all observations were 38.2° and 41.4°C. The mean was 39.68°C. The variability range within which two-thirds of the normal readings should fall was calculated by statistical methods and found to be 39.4° and 39.9°C.

These figures and curves show that one temperature observation cannot be used as the norm for that rabbit. A change above or below this one reading cannot be considered to be experimentally produced unless it is great enough to fall beyond the range of normal variability. Some workers consider a steady rise or fall as experimental compared with the fluctuating normal. It is conceivable that the range would need to be determined for any given experimental environment. The extremes obtained in this series were in all cases higher than those reported by others. In no case was a normal temperature below 38°C. observed, although other investigators frequently report a minimum between 37° and 38°C. It might be well to state that the majority of my experiments were performed in California during the months from August to May. A smaller number were carried on in Idaho in the months of June and July. There was, however, no noticeable difference in the average range of temperature in the two series. In neither case, however, was the external temperature extremely low.

II. EFFECT OF EXERCISE, FOOD, SEX, POSTURE, ON THE TEMPERATURE OF THE RABBIT

A. Exercise. Kraus (11) reports that a rabbit in a treadmill showed a rise from 39.05° to 40.1°C. during seven minutes' work, the temperature returning to normal in thirty minutes. This accords with the well known effects of exercise on the temperature of man and other animals. No exact observations were made in this series; care was taken, however, in every experimental case to prevent unusual muscular movements.

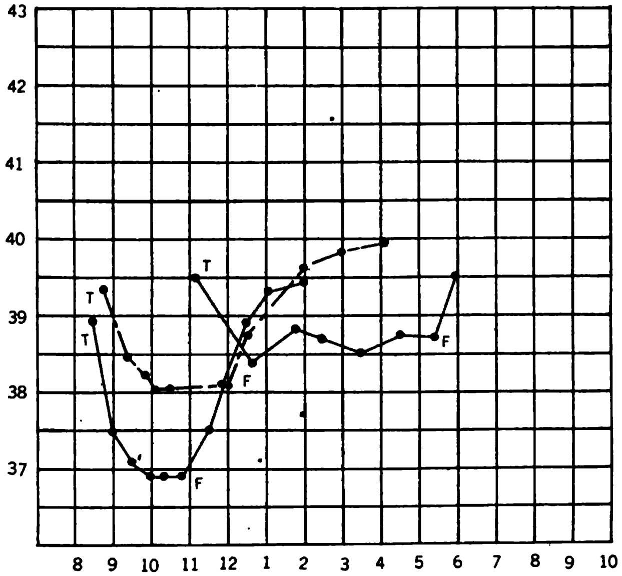

Chart 3. Temperature variations in three rabbits fastened to operating boards. Ordinates, degrees Centigrade; abscissae time in hours. *T*, tied; *F*, free.

B. Food. The rise in temperature after the taking of food is also familiar. After feeding, the rabbits used in these experiments showed a rise of 0.5°C. or more. Inanition results in a corresponding fall. The temperature of one rabbit varied from 39.5° to 32°C. during two weeks' starvation. All the animals used in this series were fed regularly and never prior to nor during an experiment, thus eliminating any possible marked deviation due to feeding.

C. Sex. It has been reported that the temperature of females often ranges slightly above that of the males. The observations on normal rabbits cited above tend to confirm this.

D. Posture. It is well known that the temperature of rabbits falls when they are tied down. Kraus (11) noted a fall of 0.2° to 0.4°C. in five to ten minutes. Chart 3 gives the temperature changes which I found in rabbits fastened to an operating board, back uppermost, for varying length of time. The eight cases recorded gave a fall of 1° to 2°C. in one and one-half to two hours. After reaching the minimum, the temperature remained practically stationary as long as the rabbits were kept in that position, except that occasionally struggling caused a rise of 0.2° to 0.3°C.

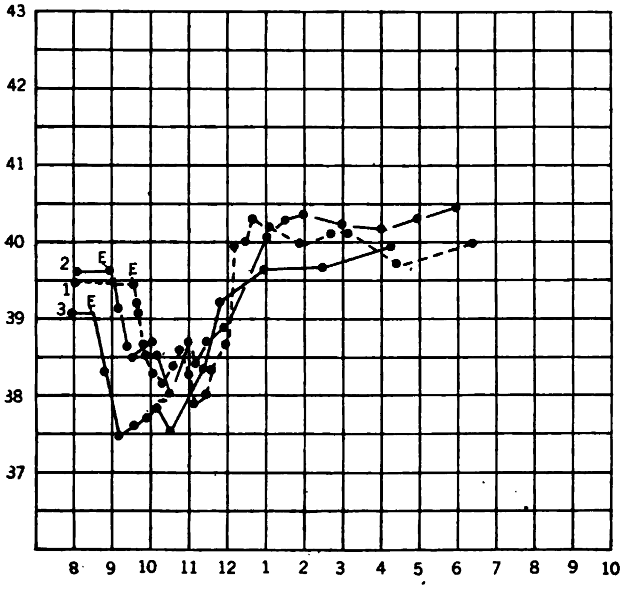

Chart 4. Temperature variations due to ether anaesthesia. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 6, ether administered for twenty minutes; *2*, rabbit 5, ether administered for twenty minutes; *3*, rabbit 4, ether administered for five minutes. *E*, Ether.

It would seem advisable in cases in which it is necessary to keep a rabbit extended for any length of time, to obtain this minimum temperature before attempting to secure experimental changes. After releasing the rabbits, the temperature rose generally 0.1 to 0.5°C. higher than at the beginning of the experiment. The extremes were 36.95° and 39.7°C.

III. EFFECTS OF ANAESTHETICS ON THE TEMPERATURE OF THE RABBIT

Anaesthetics in general are reported as causing a lowering of body temperature during and following their administration. Ether, according to Angelesco (12) causes a fall until the animal "comes out," and then a rise. The fall is due to vasodilatation and lessened muscular activity and tonus. Hale White (9) as preliminary to experiments on the "heat centers" tested the effect of ether and concluded that it did not cause an abnormal temperature. Most workers, however, agree that there is a fall in ether anaesthesia amounting often to as much as 5°C.

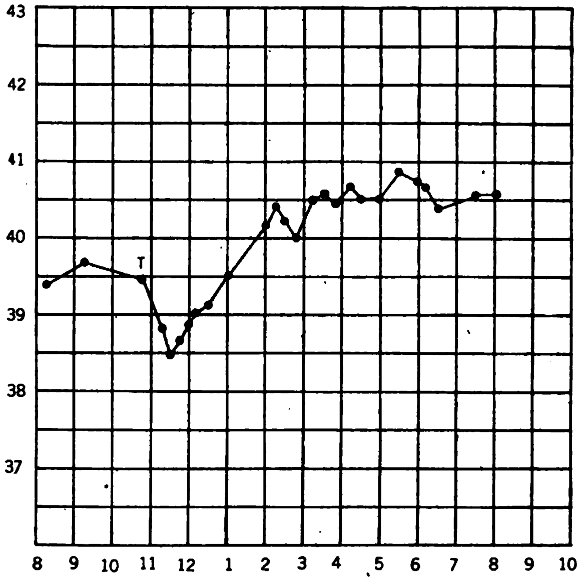

Chart 5. Temperature variations following trephining of the skull under nitrous oxide anaesthesia. Ordinates, degrees Centigrade; abscissae, time in hours. *T*, trephine.

Inconsistencies in the results obtained in experimental work in this laboratory in relation to temperature led to a reinvestigation of the effects of ether and nitrous oxide on body temperature. Chart 4 gives specimen curves of the temperature variations due to ether anaesthesia. It will be noted that there is a fall of 1.5°C. in the first hour after the cessation of etherization, followed by a rise of 1°C. above the initial temperature in the subsequent two hours. The extremes were 37.4° and 40.9°C. Most writers fail to report the final rise. Nitrous oxide gas gave similar changes but within narrower limits. In ten other observations on the effects of ether and of nitrous oxide on temperature and in some two hundred operative experiments on rabbits in which ether or nitrous oxide was used as an anaesthetic similar changes were noted.

IV. THE EFFECT OF OPFRATIVE PROCEDURE ON THE TEMPERATURE OF THE RABBIT

Hale White (9) reports dummy experiments in which trephine openings were made in the skull. In some cases the white matter was injured. He finds abnormal temperatures in only a few cases. His readings, however, were taken at intervals of several hours so that if high temperatures occurred he might easily have failed to observe them.

In three rabbits in this series trephine openings were made in the skull under ether or nitrous oxide anaesthesia. They all showed an initial fall of 1° to 2°C. followed by a rise of more than 1°C. above normal. The extremes were 36.9° and 40.9°C. Chart 5 gives a specimen curve of the above.

SUMMARY

1. The normal range of variability in the temperature of the rabbits used, including daily variations, was found to be between 39.4° and 39.9°C. with an average of 39.68°C. and extremes of 38.2° and 41.4°C.
2. Anaesthetics necessary in operative procedure cause a marked variation in the temperature of the rabbit; ether an average fall of 1.4°C. followed by an average rise of 1°C. above the initial temperature; nitrous oxide gas a similar but less marked change. The extremes were 37.4° and 40.9°C.
3. The temperature of rabbits which are tied down falls 1° to 2°C. in one to two hours and remains stationary until they are released. It then rapidly rises to 0.5°C. above the initial temperature. The extremes were 36.95° and 39.7°C.
4. Operative procedure such as trephining the skull causes an aver-

age fall of 1.9°C. followed by a rise of 1°C. or more above the initial temperature. The extremes were 36.9° and 40.8°C.

CONCLUSION

Hyperthermia in rabbits cannot be considered to be experimentally produced unless it exceeds the normal and operative variations.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

II. Relation of the Corpus Striatum to the Regulation of Body Temperature

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HISTORICAL STATEMENT

A rise in temperature following injury to various parts of the brain tissue of the rabbit was long ago reported by Tscheschichin (1), Schrieber (2), Bruck and Günther (3), Eulenberg and Landois (4) and others. The rise was generally accompanied by muscular spasms. Aronsohn and Sachs (5) in 1885 first described "heat puncture" caused by puncturing or otherwise injuring or stimulating the medial side of the corpus striatum. They reported a rise of 1.7 to 2.4°C. lasting several days. These observations led to the theory that there exists in the brain of birds and mammals a special "heat center" which automatically regulates the heat production and dissipation in such a way as to maintain a constant body temperature.

Subsequent investigators affirm the existence of various other "heat centers;" Ott (6) locates the "center" in the corpus striatum, optic thalamus, tuber cinereum and pons; Girard (7) in the corpus striatum, optic thalamus, septum pellucidum and corpus callosum; Steerath (8) in the optic thalamus; Aisenstat (9), Gottlieb (10), Ito (11), Babinsky and Lehmann (12) and Nikolaides and Dontas (13) in the corpus striatum; Hale White (14) in the corpus striatum and optic thalamus; Jacobj and Roemer (15) in the lateral ventricles; Isenschmidt and Krehl (16) in the tuber cinereum; Citron and Leschke (17) somewhere above the corpora quadrigemina.

Recent work by Barbour and Wing (18), Demming (19), Prince (20) and Hashimoto (21) in Hans Meyer's laboratory in Vienna, consists in the application of pyretics and antipyretics to the "heat centers," which they find to be in the mid-ventral part of the caudate nucleus of the corpus striatum. This center they consider to be dual in nature, that is, made up of a thermogenic or heat center, and a thermolytic or cold center. Pyretics stimulate the former and depress the latter; antipyretics have a reverse action. They say, incidentally, that in some cases of puncture a fall instead of a rise in temperature was obtained. Their work is confirmed by Cloetta and Waser (22). A number of other investigators locate a special temperature regulatory "center" somewhere in the brain.

The above evidence would seem to indicate that a special "heat center" does exist in the brain. The results and conclusions are, however, in many cases open to criticism.

"Puncture fever" as described by Bruck and Günther (3), by Schrieber (2) and others was accompanied by muscular spasms, the heat production from which would need to be considered before the rise in temperature could be said to be produced by injury to a heat center.

Aronsohn and Sachs (5) did not obtain uniformly high temperatures but in many cases reported a rise of less than 1°C. (0.2 to 1°), nor were their lesions always in the corpus striatum. The medial edges of the hemispheres, the septum pellucidum and the lateral ventricles were often the seat of injury. The great length of time before the rise was obtained admits of the possibility of infection as the exciting cause. White's experiments on the optic thalamus are less convincing. When carefully analyzed, the latter's results seem to serve as negative evidence for a specific "heat center" in the corpus striatum or optic thalamus.

Barbour and Wing (18), Prince (20), Hashimoto (21) and other workers in Hans Meyer's laboratory obtained a rise in temperature only when the mid-ventral portion of the caudate nucleus of the corpus striatum was injured. In one series of twenty-five experiments, four cases showed a temperature of 41°C. or above. These were all through the mid-ventral portion of the caudate nucleus. Seven other punctures in the same limited region ranged from 39.4 to 40.9°C. which cannot be considered experimental hyperthermia. All the other punctures were through the anterior or posterior part of the caudate nucleus, and resulted in temperatures from 39.7°C. to subnormal. This evidence again tends to deny the presence of a specific heat center.

Similar criticism may be made of the results and conclusions of other investigators who describe "heat punctures."

Pembrey (23) opposed the "heat center" theory of temperature regulation, considering the centers as purely hypothetical. The compensation between heat production and heat loss brought about by physical and chemical means was, according to him, sufficient to regulate temperature (24). He found that young mammals and birds born in a well developed condition such as the guinea pig and chick, were fully active and produced enough heat to maintain a constant temperature; while helpless newly born animals such as mice and pigeons were able to regulate their temperature only to a moderate degree. In cold they could not respond by increased activity until ten or fifteen days old, at which time muscular activity came on and increased vasomotor tonus was evident. Then the temperature was regulated by heat production and heat loss with no apparent need of a heat "center."

Pembrey (25) also found that hibernating animals could awaken with an increase of body temperature after the corpus striatum had been removed. Pembrey (26) showed that the awakening of a dormouse is accompanied by violent shivering, the temperature often rising 10° to 20°C. in a few hours. Calorimetric measurements by Pembrey (27) show that the heat produced is sufficient to account for the rise without the intervenion of a heat center. Du Bois (28) showed that hibernating animals with motor paralysis had only a small rise in temperature on awakening.

Pembrey and Mutch (29) also showed that tetrahydro-β naphthylamine caused a marked rise in temperature only when violent muscular activity and convulsions resulted. If chloroform were given during the rise to prevent muscular movements the temperature fell until the effect of the chloroform had worn off. Tetrahydro-β naphthylamine also did not cause a rise in rabbits if the muscles were paralyzed by cutting the motor nerves or by curare.

Fredericq (30) found that removal of the cerebral hemispheres in pigeons produced no variations in the daily temperature curve. Corin and Van Beneden (31) obtained similar results and observed no change in the CO_2 exchange of such pigeons. Goltz's (32) well known decerebrate dog had a temperature only slightly below normal.

Du Bois (33) found that the corpus striatum, midbrain and cerebrum of marmots, pigeons and rabbits could be destroyed thus eliminating all the hypothetical heat centers without loss of temperature regulation. Mosso (34) also denies the existence of heat centers affirming that hemorrhage and excitement are the cause of the rise of temperature following punctures. He obtained a rise by the injection of cocaine after the "heat centers" were removed.

Wilson (35) from a comprehensive neurological study of the corpus striatum concludes that it cannot be termed a heat center. Injury to the corpus striatum causes hypertonicity of the muscles, often resulting in tremors sufficient to cause a marked increase in the heat production. This together with the vasoconstriction due to the increased tonus in the walls of the blood vessels could produce hyperthermia.

Sachs and Green (36) in a recent publication do not confirm the heat center theory. Lesions or stimulation of the caudate nucleus in rabbits and cats gave no greater rise than controls. Hill (37) also refutes the claim of a special heat center.

The evidence just cited points to the probability that temperature regulation is controlled entirely by factors which are not dependent on specific "heat centers" in the brain. Still we cannot as yet say why most warm-blooded animals maintain a higher level of body temperatire than the majority of cold-blooded animals. The "heat center" theory, however, conveniently accounts for this and is, therefore, still accepted by many physiologists.

The experiments reported in this paper on the relation of the corpus striatum to the regulation of body temperature were begun with the idea of applying drugs to the "heat centers" after the method of Barbour and Wing (18). The difficulty met with in locating a definite center and the variability in the results obtained by puncture, however, threw so much doubt upon the existence of such centers that the attempt was made to reinvestigate the whole matter.

EXPERIMENTS ON THE "HEAT CENTER"

A. Puncture. Since the caudate nucleus of the corpus striatum is generally accepted as the most probable "heat center," it is the only one considered in this part of my work.

The punctures were made according to the methods of Arônsohn and Sachs (5). The hair on the head of the rabbit was removed, a longitudinal incision made in the skin and a trephine opening 1 cm. in diameter made 1 mm. to the right of the longitudinal suture and 3 mm. anterior to the coronal suture. Into this opening was screwed a metal cylinder with a small central hole through which the puncture needle 1 mm. in diameter was inserted to varying depths. The needle could be removed or left in place after the puncture was made.

Aseptic precautions were used throughout the operation and the wound was covered with a sterile cotton cap held in place by flexible collodion and adhesive tape.

Ninety-four punctures were made in seventy-four rabbits. The results are given in table 1 and summarized in table 2. In sixty-two cases the temperature did not rise above 41° C. This falls within the range of variability due to anaesthetics and operative injury, which I have shown to be between 36.9° to 40.9°C. and cannot be considered as hyperthermia brought about by injury to or stimulation of a specific "heat center." Since the variability range due to incidental factors is large and since occasional temperatures above 41.4°C. are met with in normal and anaesthetized rabbits, it seems reasonable to include only those registering 41.5°C. and above in the cases of hyperthermia. This would give seventy-four cases of normal temperature to twenty of hyperthermia in the above mentioned table.

The area and location of the brain lesion varied. This was determined by means of transverse sections of the brains hardened in formalin. In thirty-seven cases of normal temperature the caudate nucleus was distinctly injured. Figure 1 gives specimen sections through the line of puncture in two of these cases. The injury to the caudate nucleus cannot be questioned. Chart 1 gives curves of the temperature following these and other similar punctures. A comparison with the curves of normal and anaesthetized rabbits shows that these temperature variations could be accounted for by other means than injury to special "centers."

In five of the cases of normal temperature following puncture of the caudate nucleus the injury was limited to the caudate nucleus; in five it was extensive with infiltration involving large areas. In the remaining cases the lesion was well defined but included with the caudate nucleus other parts as the internal capsule, lenticular nucleus, optic thalamus or infundibulum.

In eighteen cases of normal temperature there was no evident injury to the caudate nucleus (fig. 2, chart 2). In fifteen cases of normal temperature injury to the caudate nucleus was questionable as the puncture penetrated the lateral ventricle and therefore merely touched the medial edge of the caudate nucleus. They are in the table in a separate column as uncertain (fig. 3, chart 3).

In only twenty cases of punctures was a distinct hyperthermia beyond the range of variability obtained. Seven of these showed no injury to the caudate nucleus. Figure 4 is a transverse section through the point of puncture of one showing a clear line between the hemi-

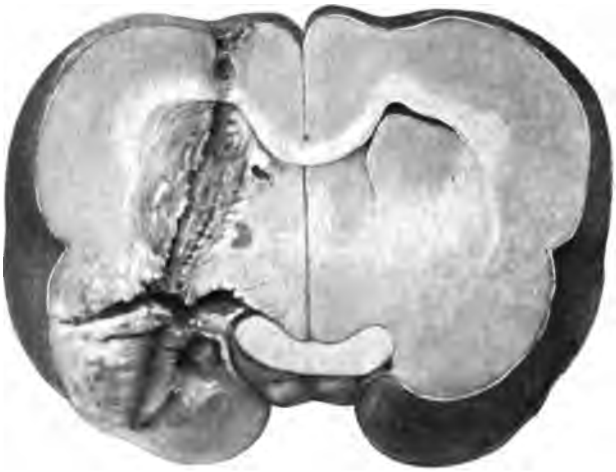

Fig. 1 a. Section of brain of rabbit 40 showing puncture through caudate nucleus; followed by normal temperature.

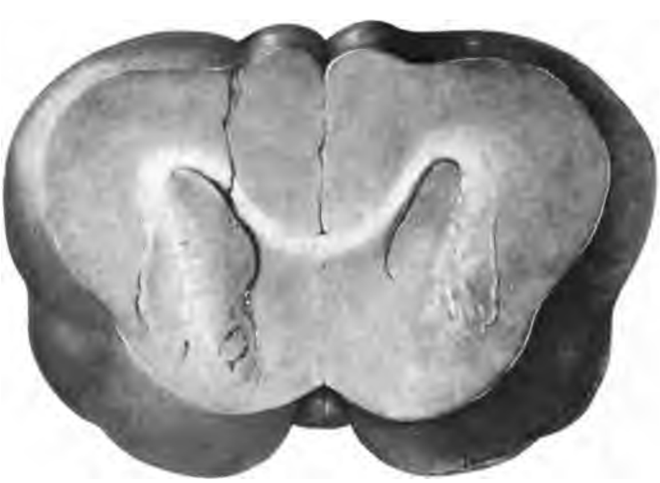

Fig. 1 b. Section of brain of rabbit 79; the puncture not wholly in one plane indicated by dotted line.

spheres with no injury to the caudate nucleus. Chart 4 gives the temperature curve of the same. Six cases had lesions (four extensive) which involved the caudate nucleus (fig. 5, chart 5). Six cases were doubtful.

Table 2 shows that 85 per cent of the punctures involving the caudate nucleus failed to produce a rise in temperature above 41.5°C. Only 15 per cent gave distinct hyperthermia.

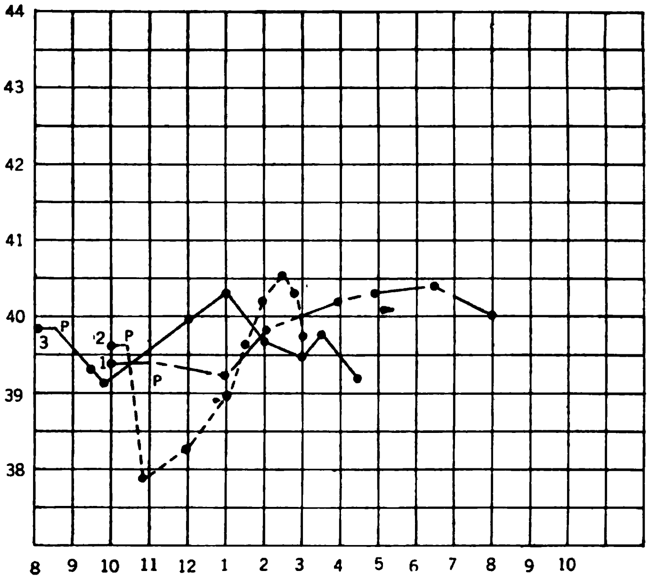

Chart 1. Specimen curves of normal temperature following puncture of caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 59; *2*, rabbit 79; *3*, rabbit 27, *4*, rabbit 40. *P*, Puncture.

In the above experiments all cases of hyperthermia were associated with excessive muscular movements often taking the form of clonic convulsions. Calorimetric measurements of the heat production were not taken but from the usual rise accompanying muscular exercise it seems reasonable to assume that the violent movements were sufficient to account for the hyperthermia. Exact data on this phase of the subject will be obtained in later investigations.

In another series of experiments punctures were made with a "heating and cooling cylinder" similar to the one used by Barbour (38) for the purpose of heating and cooling the caudate nucleus. The needle

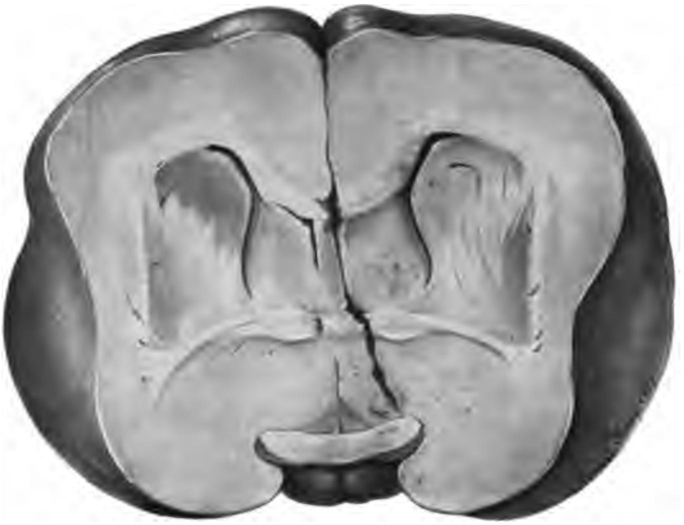

Fig. 2. Section of brain of rabbit 67; showing puncture line between the hemispheres with no injury to the caudate nucleus. A normal temperature followed.

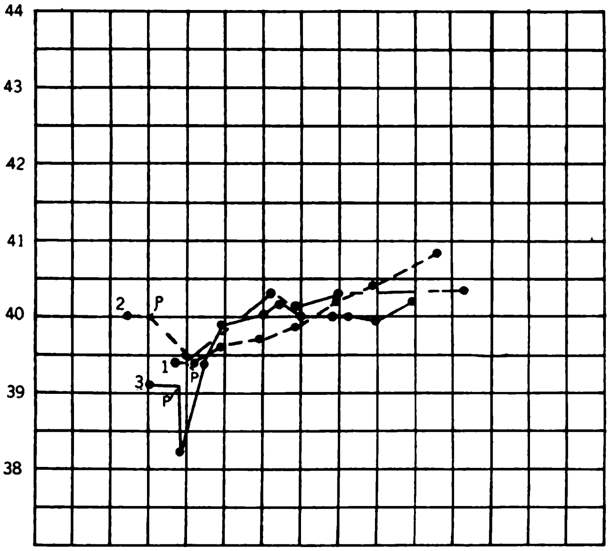

Chart 2. Specimen curves of normal temperature following punctures with no injury to the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 67; *2*, rabbit 73; *3*, rabbit 17. *P*, Puncture.

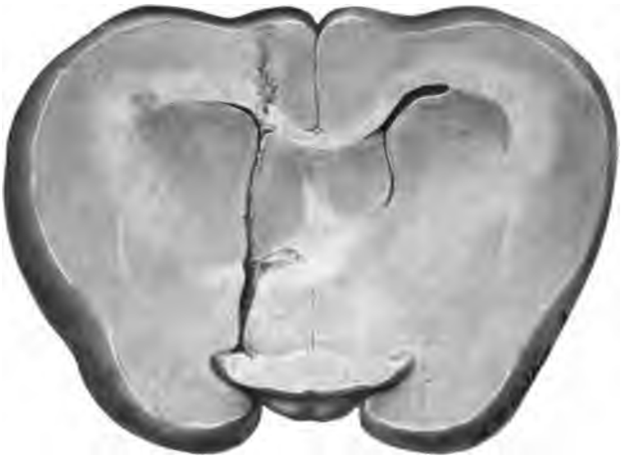

Fig. 3 a. Section of brain of rabbit 71 showing puncture through lateral ventricle with possible injury to caudate nucleus; followed by hyperthermia.

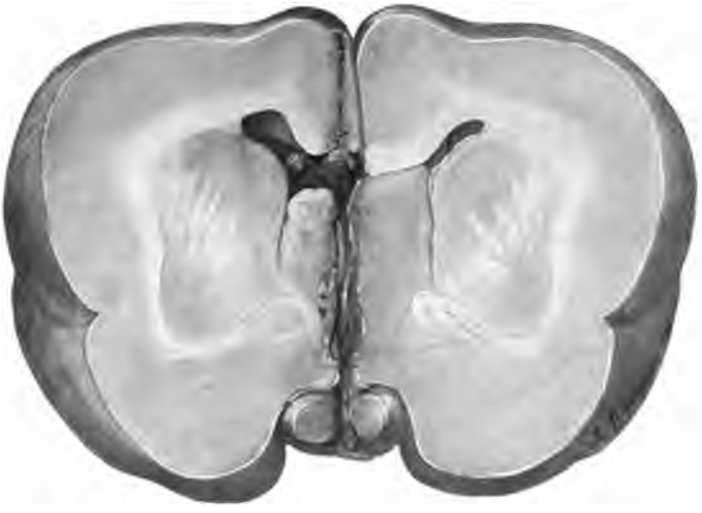

Fig. 3 b. Section of brain of rabbit 47 showing puncture through lateral ventricle; followed by normal temperature.

was 3 to 4 mm. in diameter instead of 1 mm. as in the first series; only five of the thirteen cases showed hyperthermia which was, in every case, preceded and accompanied by violent muscular movements and convulsions; six died within the course of a few hours. The cause of sudden death in these and other cases is being investigated further. While the above results seem to indicate that hyperthermia does in certain cases follow puncture of the brain of the rabbit, it cannot be said that the rise in temperature depends on injury to the caudate nucleus nor can the rise be ascribed to any other definite "center" since there is no apparent correlation between the location of the lesion and the occurrence of hyperthermia. It can be concluded, in fact, that injury to the caudate nucleus or other alleged "heat centers" and "puncture fever" bear no close relation to each other.

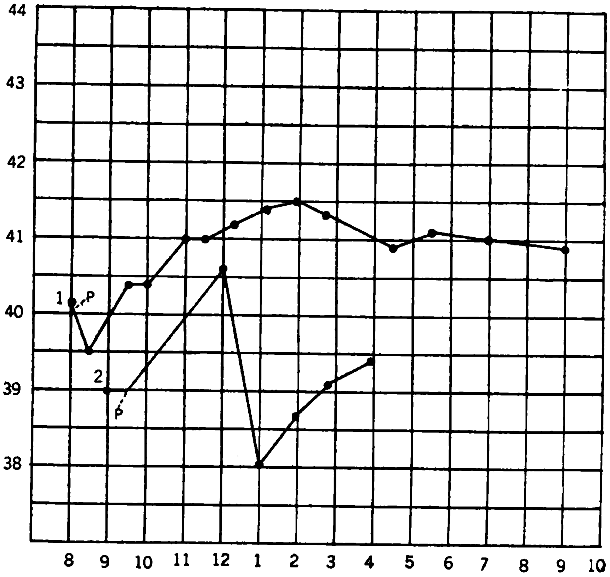

Chart 3. Specimen curves of temperature variations following punctures in the lateral ventricle. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 71; *2*, rabbit 47. *P*, Puncture.

B. Application of pyretics and antipyretics. The caudate nucleus was heated and cooled according to the method of Barbour (38) and of Hashimoto (21) by means of a metal cylinder through which hot or cold water could be passed at will. Heating caused a fall in temperature of 0.2° to 1.4°C. Cooling a rise of 0.5° to 1.6°C. Table 3 gives the

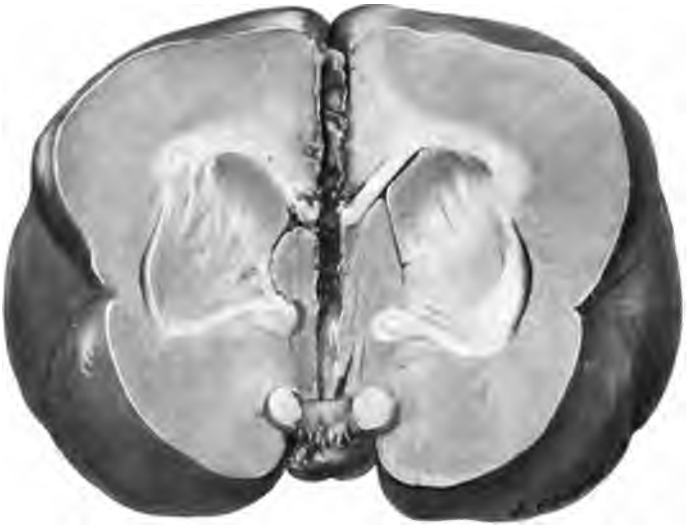

Fig. 4. Section of the brain of rabbit 22 showing puncture line between the hemispheres with no injury to the caudate nucleus. Hyperthermia followed the puncture.

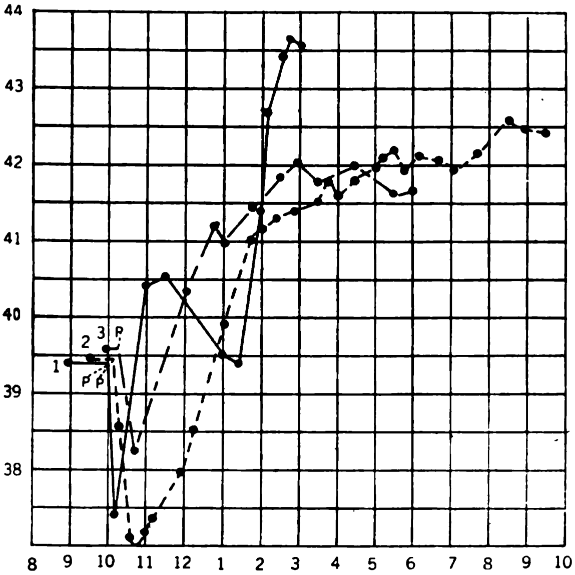

Chart 4. Specimen curves of hyperthermia following punctures with no injury to the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 22; *2*, rabbit 10; *3*, rabbit 29. *P*, Puncture.

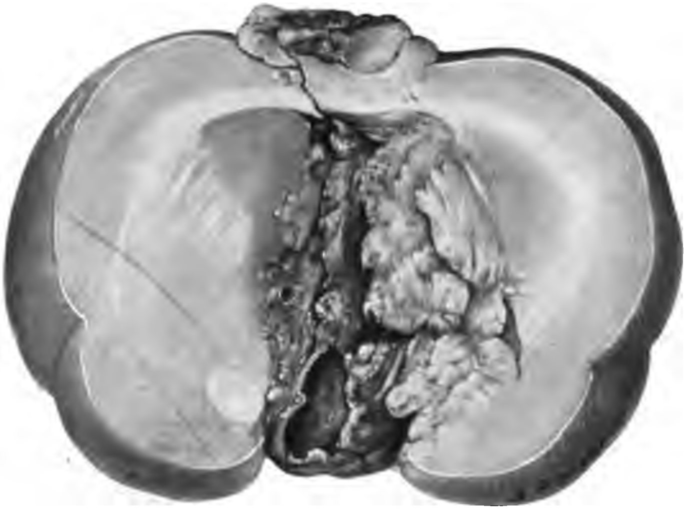

Fig. 5 a. Section of brain of rabbit 61 showing extensive lesion involving caudate nucleus; followed by hyperthermia.

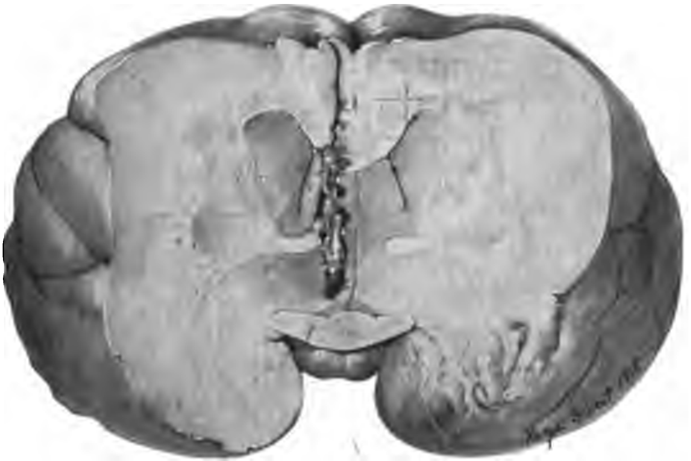

Fig. 5 b. Section of brain of rabbit 51 showing slight injury to caudate nucleus; followed by hyperthermia.

results. They accord with those of Barbour (38) and of Hashimotor (21) but might conceivably be due to an indirect or direct effect on the vasomotor centers in the medulla. The fact that heating and cooling the medulla (experiments to be described later) gave similar results tends to show that this may be the case.

An attempt was made to apply drugs to the caudate nucleus, as Barbour and Wing (18) had done, by injecting into the puncture hole. Barbour states that there was often an overflow of ventricular fluid and drug. The same difficulty was met in my experiments. In every case the pressure of the fluid was sufficient to render it very doubtful whether any of the drug reached the brain tissue, especially the caudate nucleus. A more exact method of applying drugs to parts of the brain below the cortex should be devised.

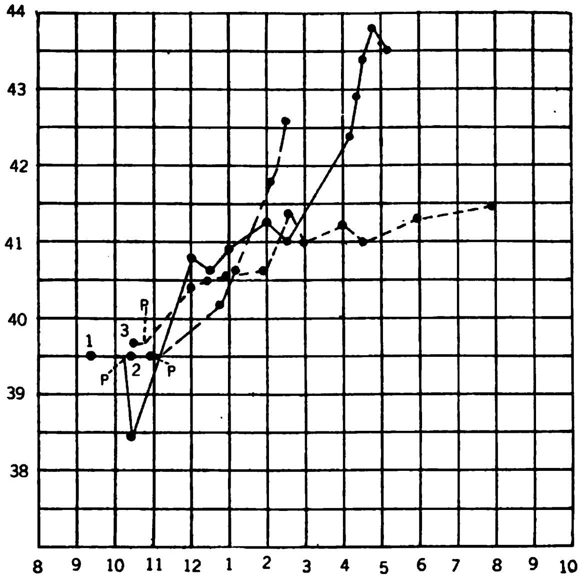

Chart 5. Specimen curves of hyperthermia following puncture of the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 20; *2*, rabbit 61; *3*, rabbit 51. *P*, Puncture.

C. Removal. With aseptic precautions a large area of the brain was exposed by removing the skull with a trephine and bone forceps. Bleeding was stopped by pressure with sterile cotton. The cortex was lifted off with a large curette, thus leaving the caudate nuclei clearly exposed to view. They were then carefully removed with a

TABLE 1

Effect of puncture on the temperature of the rabbit

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1916				*hours*	
1	November 13	No autopsy	39.4	38.4	7	
2	November 17	Lateral ventricle, *caudate nucleus doubtful*	39.3	39.2	5	
3	November 21	Lateral ventricle, optic thalamus, infundibulum, *caudate nucleus doubtful*	38.5	39.5	8	
3	November 25	Same as on 21st	39.4	40.1	7	
3	November 27	Left lenticular nucleus	39.4	40.2	6	
4	November 30	Lateral ventricle, optic thalamus, infundibulum	39.8	40.1	4	
4	December 3	Lateral ventricle, optic thalamus, infundibulum	39.9	40.7	3	
7	December 1	Lateral ventricle, *caudate nucleus*	40.1	39.9	3	
7	December 3	Lateral ventricle, *caudate nucleus*	39.1	40.45	5	
8	November 17	Lateral ventricle, *caudate nucleus* doubtful	39.5	40.6	2	
8	November 20	Lateral ventricle, *caudate nucleus* doubtful	39.6	40.1	2½	
8	November 21	Lateral ventricle, *caudate nucleus* doubtful	39.6	40.0	1	
8	November 24	Lateral ventricle, *caudate nucleus* doubtful	39.1	39.5	3	
10	December 19	Between hemispheres, optic chiasma	39.4	*42.2*	7	
14	December 30	*Caudate nucleus;* lenticular nucleus	39.1	41.1	6	
14	December 31	*Caudate nucleus;* lenticular nucleus	39.1	40.9	6	
15	December 30	Lateral ventricle, infundibulum	39.2	*41.5*	4½	
16	December 22	Lateral ventricle, optic thalamus, extensive infiltration, caudate nucleus doubtful	38.8	40.6	6½	

16	December 29	Same as on 22d	38.7	39.5	1½	
16	December 30	Same as on 22d	39.2	*41.5*	1½	
16	December 31	Same as on 22d	40.7	39.7	1	
	1917					
17	January 25	Between hemispheres, infundibulum	39.1	40.2	3	
17	January 26	Between hemispheres, infundibulum	39.3	40.3	5	Died on day of operation
18	January 25	No autopsy	39.4	40.1	10	
18	January 26	No autopsy	39.3	*43.0*	5	Convulsions
18	January 27	No autopsy	39.5	39.2	6	Died on day of puncture
19	January 25	Lateral ventricle, optic thalamus, infundibulum, caudate nucleus doubtful	39.5	41.4	7	
19	January 26	Same as on 25th	40.3	41.0	7	
19	January 27	Same as on 25th	39.5	40.4	3	
20	January 31	Extensive infiltration involving caudate nucleus, optic thalamus, infundibulum	39.5	43.8	7	Moved about continually and rapidly. Tremors. Died on day of puncture
21	January 30	Same as no. 20	39.6	41.3	8	
22	January 29	Between hemispheres, infundibulum, optic chiasma	39.4	*43.65*	5	Constant movement and tremors. Died on day of puncture
23	February 3	Lateral ventricle, infundibulum, optic thalamus	39.5	39.5	5	
23	February 5	Lateral ventricle, infundibulum, optic thalamus	39.6	41.1	9	
23	February 6	Lateral ventricle, infundibulum, optic thalamus	40.1	37.1	3	Died on day of puncture
25	February 7	Extensive optic thalamus, infundibulum, *caudate nucleus*	39.7	39.3	1½	Died on day of puncture
26	February 7	Lateral ventricle, optic thalamus, *caudate nucleus*	39.7	41.5	6	Died on day of puncture

TABLE 1—*Continued*

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1917				*hours*	
27	February 8	Lateral ventricle, infundibulum, optic thalamus, *caudate nucleus*	39.6	40.5	4	Died on day of puncture
28	February 10	Lateral ventricle, infundibulum, optic thalamus, *caudate nucleus doubtful*	39.3	41.0	4	Moved continually, died on day of puncture
29	February 13	Septum pellucidum, infundibulum	39.5	*42.0*	5	
29	February 15	Left side, through cortex only	37.6	37.2	5	Convulsions. Died on day of puncture
33	February 19	Lateral ventricle, whole *corpus striatum*	39.5	41.4	6	Convulsions. Died on day of puncture
35	February 20	Lateral ventricle whole *corpus striatum*	39.8	41.3	7	
36	February 21	Lateral ventricle, *caudate nucleus* infundibulum	39.6	41.4	7	
37	February 22	Lateral ventricle, *caudate nucleus*, lenticular nucleus	39.2	40.5	8	
38	February 28	Between hemispheres, optic chiasma	39.6	41.7	10	Continual movement. Died on day of puncture
39	February 28	Extensive, caudate nucleus, optic thalamus, infundibulum	36.6	41.0	10	
39	March 1	Same as on February 28	41.0	39.7	8	Died on day of puncture
40	March 1	Lateral ventricle, caudate and lenticular nucleus	39.8	40.3	10	Died on day of puncture
41	March 1	Lateral ventricle, caudate nucleus, infundibulum optic thalamus	39.4	40.3	11	

41	March 2	Lateral ventricle, caudate nucleus; infundibulum optic thalamus	40.3	41.3	3	
42	March 5	Lateral ventricle, infundibulum, optic thalamus	38.9	40.4	7	Convulsions; died on day of puncture
43	March 5	Lateral ventricle caudate nucleus, optic chiasma	39.6	40.5	5	
43	March 6	Lateral ventricle, caudate nucleus, infundibulum	39.7	*43.2*	8	Frantic movements. Died on day of puncture
44	March 6	Lateral ventricle, *caudate nucleus doubtful*	39.2	38.8	4	Died in four hours
45	March 7	Lateral ventricle, *caudate nucleus*, infundibulum, optic thalamus, extensive	39.8	*41.8*	4	
46	March 10	Lateral ventricle, *caudate nucleus*, infundibulum, optic thalamus, extensive	38.5	*42.0*	3½	Frantic movements and convulsions. Died on day of puncture
47	March 12	Lateral ventricle, *caudate nucleus, doubtful,* infundibulum, optic thalamus, extensive	39.0	39.4	6	
48	April 30	Lateral ventricle, *caudate nucleus, doubtful,* infundibulum	39.8	*42.6*	10	Frantic movements, convulsions; died on day of puncture
49	May 2	Lateral ventricle, *caudate nucleus*	39.0			Died in two hours
50	May 3	Lateral ventricle, *caudate nucleus*, infundibulum, optic thalamus	39.6	39.8	6	
51	May 4	Lateral ventricle, *caudate nucleus*, slight	39.6	*41.5*	9	
52	May 5	Lateral ventricle, *caudate nucleus,*	39.2	40.8	1	
53	May 6	Lateral ventricle, *caudate nucleus*, infundibulum, optic thalamus	39.1	40.3	5	
53b	May 6	Lateral ventricle, *caudate nucleus*	39.2	40.1	4	Convulsions. Died on day of puncture
54	April 30	Lateral ventricle, caudate nucleus, optic thalamus	38.8	39.5	7	

TABLE 1—*Continued*

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1916				*hours*	
55	April 30	Lateral ventricle	39.9	41.2	3	Sick
56	May 1	Lateral ventricle, infundibulum, optic thalamus	40.0	*42.2*	2	Constant rapid movement
57	May 1	Lateral ventricle, caudate nucleus, optic thalamus, infundibulum	40.1	41.2	5	Died on day of puncture
58	May 2	Between hemispheres	39.3	40.5	4	
59	May 3	*Caudate nucleus*, internal capsule, optic thalamus	39.5	40.6	3	
60	May 6	*Caudate nucleus* slightly, lateral ventricle	38.8	40.8	6	
61	May 6	*Caudate nucleus* extensive, infundibulum	39.5	42.55	4	Continual movement, convulsions; died on day of puncture
62	May 7	*Caudate nucleus doubtful*, corpus callosum	39.5	*41.5*	4	
63	May 7	*Caudate nucleus*, lenticular nucleus	39.3	40.0	7	
64	May 8	Between hemispheres, infundibulum	39.3	41.1	5	
65	May 8	To corpus callosum	39.5	41.0	7	
66	May 8	Extensive, caudate nucleus, optic thalamus, infundibulum	39.6	40.9	3	Died on day of puncture
67	May 8	Between hemispheres to optic chiasma	39.5	40.3	4	
68	June 19	*Caudate nucleus* doubtful, infundibulum	40.0	*42.3*	6	Very restless
68	June 20	*Caudate nucleus* doubtful, infundibulum		*42.0*	2	
69	June 21	*Caudate nucleus*, lateral ventricle, infundibulum, optic thalamus	39.65	38.8	3	

70	June 22	*Caudate nucleus*, lateral ventricle, infundibulum, optic thalamus	39.4	40.2	5	Slight convulsions. Died on day of puncture
71	July 3	*Caudate nucleus* doubtful, lateral ventricle, optic chiasma	40.0	41.5	5	
72	July 5	Lateral ventricle, optic thalamus	39.0	40.5	7	
73	July 12	Optic thalamus, fornix, lenticular nucleus	40.1	40.8	6	Died on day of puncture
74	July 14	Optic thalamus, fornix, infundibulum	39.5	*42.0*	6	
75	July 15	Optic thalamus, fornix, lenticular nucleus	40.0	40.6	6	
76	July 20	Caudate nucleus, lenticular nucleus, internal capsule	39.5	40.8	7	
77	July 25	Extensive, caudate and lenticular nuclei, optic thalamus, infundibulum	30.2			Died in convulsions in 1½ hours
78	July 26	Lateral ventricle, caudate nucleus slightly, optic thalamus, infundibulum	39.3	40.4	3	Died on day of puncture
80	August 2	No autopsy		40.3	5	
81	August 5	Between hemispheres		41.4	5	
82	August 6	Between hemispheres		39.0	5	

TABLE 2

Summary of effect of puncture on the temperature of the rabbit

LESION	NUMBER OF PUNCTURES			
	Hyperthermia		NORMAL TEMPERATURE	
	Above 41.5°C.	Above 41.°C.	Below 41.5°C.	Below 41.°C.
Involving caudate nucleus	*6*	13	*37*	30
Not involving caudate nucleus	*7*	11	*18*	14
Possible injury to caudate nucleus	*6*	7	*15*	14
No autopsy	1	1	4	4

TABLE 3

The effect of heating and cooling the caudate nucleus

NUMBER OF RABBIT	DATE	TEMPERATURE			DURATION OF PASSAGE OF WATER
		Before passage of water	After passage of water, 45-50°C.	Amount of change °C.	
	1917				*hours*
100	November 14	40.5	39.4	−1.1	1½
105	November 21	39.9	38.5	−1.4	1
120	December 10	41.2	39.4	−1.8	2½
	1918				
123	January 30	41.1	40.5	−0.6	1
	January 31	40.5	40.9	+0.4	½
126	February 2	38.7	38.5	−0.2	1
	February 3	38.5	38.7	+0.2	½
			Water 15 –20°C.		
	1917				
105	December 21	40.5	39.9	−0.5	1
	1918				
123	January 30	41.0	41.5	+0.5	½
	January 31	40.9	41.5	+0.6	1
126	February 2	38.9	40.5	+1.6	2

small curette. The skin on the head was then replaced and fastened together and the wound bandaged with cotton and flexible collodion and adhesive tape. The rabbits survived the operation several days. Some were killed on the third day.

Eighteen operations were performed and in every case a normal temperature was maintained. Careful autopsies by means of transverse sections of the brains hardened in formalin were made. In seven brains no trace of the caudate nuclei remained, in ten a portion 1 mm.

or less in diameter of the posterior tip was intact but could have had no connection with any other portion of the brain nor with the cord.

Similar experiments were made on pigeons. Since the corpus striatum makes up the major part of the forebrain, both cerebral hemispheres were removed. The same results were obtained as for rabbits, that is, a normal temperature was maintained subsequent to the operation in every case. Physiological behavior as well as autopsy findings indicated that the cerebral hemispheres had been completely removed.

These results indicate that a normal body temperature in rabbits and pigeons can be maintained without the aid of the caudate nucleus of the corpus striatum.

SUMMARY

1. Seventy-eight per cent of all the punctures failed to produce an abnormally high temperature. Of the 22 per cent of cases in which hyperthermia was obtained, only one-third showed injury to the caudate nucleus. Approximately one-half of the punctures were distinctly through the caudate nucleus; 85 per cent of these, however, were not followed by hyperthermia.
2. Heating the caudate nucleus caused a slight fall in temperature, cooling a slight rise. In this respect the results agree with those of Barbour.
3. After removal of the caudate nucleus in rabbits and the cerebral hemispheres in pigeons, a normal body temperature was maintained.

CONCLUSIONS

The corpora striata are not essential for the maintenance of a constant body temperature since their puncture in rabbits or their removal in rabbits and pigeons does not alter the normal temperature.

The existence of special "heat centers" in the brain is therefore not confirmed.

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THE NURSING MOTHER AS A FACTOR OF SAFETY IN THE NUTRITION OF THE YOUNG[1]

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THE ESSENTIAL CONSTITUENTS OF AN ADEQUATE DIET

Since the solution of the main problems involved in the successful feeding of simplified diets which consisted of purified substances, progress in the advancement of our knowledge of nutrition has been rapid. It has become evident that among the thousand or more chemical substances which occur in animal and plant tissues it is essential that the diet of the mammal shall contain only the following:

Sixteen or seventeen amino-acids which result from the digestion of the complete proteins;[2] the carbohydrate glucose or one of its polysaccharides (starch, dextrine, etc., or other sugar which in the body is convertible into glucose); probably nine inorganic elements in the form of suitable compounds (Ca, Mg, Na, K, Cl, P, I, Fe, S), and two as yet chemically unidentified dietary essentials, fat-soluble A[3] and water-soluble B. Such a mixture may be capable of supporting normal nutrition throughout the life of an animal beyond the weaning period.

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[1] A few of the experiments described in this paper were carried out by the authors at the Wisconsin Experiment Station.

[2] So far as has been definitely established all the amino-acids which the "complete" proteins yield on hydrolysis must with the exception of glycocoll be supplied in the food mixture. The suggestive experiments of Hopkins (1) seem to indicate that several of the aliphatic amino-acids are dispensable from the diet, but the feeding periods were, in our opinion, too brief to make the results conclusive.

[3] Although it has not been found possible to successfully nourish animals on diets free from lipoids, there is much evidence that not only fats and lecithins, but the other complex lipoids as well can be synthetically produced by the animal tissues (2). It seems probable that if it were possible to supply fat-soluble A without at the same time adding fats, young animals could be satisfactorily nourished on lipoid-free diets.

The terms fat-soluble A and water-soluble B were introduced by McCollum and Kennedy to designate two chemical substances, or so far as could be determined at that time, possibly groups of substances, both of which are indispensable in the diet (3). Subsequent developments have made it clear that at least in the case of the water-soluble B but one substance indispensable for the maintenance of physiological well being is involved (4). In the case of the dietary factor fat-soluble A, it is possible that more than a single indispensable substance is present in the simplest food preparations which furnish it but there is not the slightest evidence that there is more than one. Fat-soluble A is the dietary factor of unknown chemical nature, the absence of which from the diet leads to the development of a peculiar condition of the eyes. The eyelids and tissues surrounding the eyes swell so that the eyes cannot be opened or are opened with difficulty. There is inflammation of the cornea, and if the missing dietary essential is not promptly supplied, permanent blindness ensues. This disease is a type of xerophthalmia (5). Butter fat, milk and the fats of egg yolk are the best sources of the substance, fat-soluble A, but it is also present to the extent of about three times the food requirements of the growing animal in the leaves of such plants as spinach, celery (tops), alfalfa and other leaves which are not fleshy and which dry easily when separated from the plant. This is shown by the fact that 30 per cent of one of these leaves in the diet supplies enough of this substance to maintain an animal in a normal condition, provided the rest of the diet is properly constituted (6). Fleshy leaves such as the cabbage, which are in some degree modified as storage organs, contain proportionately less of the substance, fat-soluble A. Seeds and seed products contain in general much less of the substance than do the leaves. This difference in the content of different foods in the dietary essential, fat-soluble A, depends upon the extent to which the foodstuffs consist of cellular elements as contrasted with reserve food materials (protein, starch, sugars, fats and inorganic salts). The substance is associated with the germ and with the limited areas of the seed which consist of cellular elements, rather than with the endosperm (7).

The second dietary essential of unknown chemical nature, water-soluble B, is much more abundant in nature than is fat-soluble A. It too is associated more abundantly with the cells of the animal and plant tissues rather than with the reserve food supply of the seed. A lack of this substance in the diet leads to the development of a condition of polyneuritis which in man is known as beriberi. There is almost conclusive evidence that the preparations which contain this substance in concentrated form contain but a single indispensable chemical substance of unknown nature. This view is borne out by the fact that beriberi and the type of xerophthalmia of dietary origin are the only diseases referable to faulty diet, for which "curative" substances exist. Other diseases, especially scurvy and pellagra, have been referred by Funk (8) and others to the group of so-called deficiency diseases, in the same sense as the syndromes beriberi and xerophthalmia, i.e., the assumption is made that for each of them there exists a "curative" substance.

Our knowledge concerning the degree to which the diet of the higher animals can be simplified without interfering with their normal development, is the result of a long series of feeding experiments with purified foodstuffs carried out by McCollum and Davis (9). It has a scientific interest in that it makes clear the great extent of the synthetic power of the animal body, but a very much greater value in that it afforded the key to the solution of the greatest problem of the human race, viz., the problem of approximating the optimum in the character of the food which we consume. For half a century the energy and protein content have been the criteria by which dietitians have attempted to judge the quality of food mixtures. In a land of plenty, where a wide variety of foodstuffs is available, dairy products, meats and the produce of the vegetable garden, as well as the staple cereals and potatoes are within the reach of even the poorest people, it is not strange that the inadequacy of such a basis for the estimation of the quality of rations should long escape criticism. Only in the field of animal production, where rations monotonous in character and restricted as to source to one, two or three foodstuffs, were fed week after week, did it become apparent that the data afforded by chemical analysis failed to disclose the quality of a food mixture as made manifest by its power to induce rapid growth in the young, vigor and high fertility in the adult, and the capacity to produce strong offspring and an abundance of milk for their nutrition. That this fact was recognized by shrewd animal husbandrymen is made evident by the fact that although standard works on the feeding of farm animals continue to the present day to discuss food values on the basis of crude protein content, energy value and digestibility, agricultural experiment stations have for years been testing by feeding experiments the value of this versus that protein concentrate as a supplement for each of the more important feeds grown on the farm. If the proteins from one source were as good as those from another, and the digestibility were equally high, such comparative feeding trials should be unnecessary and the feeding value of several mixtures which would give similar results when subjected to chemical analysis should be equal. This, however, is not the case. Feeders have long recognized the superiority of milk and buttermilk over any of the by-products of the milling industry, as supplements to rations derived from seeds and seed products.

The nature of the dietary deficiencies of the seeds of plants. Once in possession of the knowledge concerning just what factors operate to make an adequate diet, McCollum and Davis, McCollum, Simmonds and Pitz, and McCollum and Simmonds were able to show by the systematic feeding of a single natural foodstuff, as wheat (10), maize (11), rolled oats (12), rice (13), wheat germ (14), pea (6), navy bean (15), kaffir corn (16), (17), rye and barley (17), with single and multiple purified food additions, just what kind of deficiencies are responsible for the failures which had long been known to result from the feeding of certain diets which were greatly restricted as to source. The soy bean has been studied by Daniels and Nichols (18) and also by Osborne and Mendel (19), and the cottonseed by Richardson and Green (20).

It was a great surprise to find that such a mixture of seeds as whole wheat, rolled oats, whole corn, unpolished rice, etc., when fed as the sole source of nutriment fail utterly to induce any growth in young animals or to long maintain life. The reason for this we have shown in the papers cited.

These investigations made it clear that the seeds as a class are deficient in the same respect, viz., they are all too poor in three inorganic elements, calcium, sodium and chlorine, to permit an animal to grow. It was shown that each of these seeds is not enhanced by the addition of any other inorganic elements than those mentioned (21). Each of these seeds, with the exception of millet seed (22), is too low in its content of the fat-soluble A to maintain an animal in a good state of health over a long period, and the quality of the protein in each is of relatively low biological value and must be supplemented by the addition of protein before growth at the maximum rate can be secured. The seeds are therefore to be classed together as a distinct group of natural foods having the same limitations from the dietary standpoint. For an appreciation of the problems relating to the diet during lactation which we present in this paper, it is essential that the following facts be fully appreciated:

1. Young animals cannot grow when limited to a single seed or mixture of seeds as their sole source of nutriment, with no accidental supply of mineral salts in the drinking water.
2. Young animals cannot grow when fed a single seed or mixture of seeds, even though the latter is supplemented with purified protein and a fat containing fat-soluble A. The inorganic content is the first limiting factor and sodium, chlorine and calcium must be added before growth becomes possible.
3. The proteins of the seeds, and their content of fat-soluble A, as well as all other dietary factors, are of such a value as will permit young animals to grow for a considerable time, and remain in apparent good health when the diet consists of one or more seeds, supplemented with the necessary inorganic salts. On such a diet faulty nutrition is first observed only after the lapse of a considerable time.

DIFFERENCES IN THE COMPOSITION OF THE MILK AS THE RESULT OF THE QUALITY OF THE DIET OF THE MOTHER

The extent to which the maternal organism through the secretion of the mammary gland can serve as a factor of safety for the suckling, is still very little understood. It is well known that the proteins of milk are of distinctly higher quality for the promotion of growth than are those of the vegetable foods generally. This is shown by the fact that young animals grow better on 6 or 7 per cent of milk protein (23) than on higher intakes of plant proteins (24). In respect to the protein factor, therefore, the maternal organism selects certain of the amino-acids from among the digestion products of the food and presents them in the milk for the nutrition of the young in such proportions as make possible a very efficient transformation into the body proteins of the young. She makes from her large intake of protein of rather poor quality a smaller output of protein of exceptional biological value in the milk

The importance of this service of the mother to the young in supplying it a protein mixture suitable for efficient utilization for growth, will doubtless depend upon the character of the mother's food. It is well known that certain amino-acids greatly stimulate metabolism because of their "specific dynamic action" (25), and lead to increased heat production and increased carbon dioxide output. Beyond a certain limit, not well defined, this would be of course a useless waste of energy and would tend to interfere with the formation and with the storage of new tissue. It is like keeping the furnace going in warm weather. In former papers we attempted to throw light on the question as to whether the young animal is better off with the minimum supply of protein of high biological value, necessary for normal growth, than it is with food proteins of low value for growth when the latter are fed at such high planes of intake that the same biological value for growth would be reached or surpassed. Wheat proteins (24) were employed in the one series of experiments and milk proteins in the other (23). The animals were able to grow better on 6 to 8 per cent of milk proteins than on any plane of wheat proteins up to over 40 per cent of the food mixture. The results were not considered conclusive, however, in giving an answer to the question in point because it appears that there is something detrimental about the wheat products which may have depressed the vitality of the animals. Final deductions cannot be drawn until these experiments are repeated using proteins of low value but without detrimental qualities other than such as may come from the disposal by the organism of the excess of useless amino-acids which cannot, because of certain essential ones being nearly absent, be built up into tissue proteins.

The lactating mother certainly concentrates in the milk she produces much, if not all, of the dietary essential fat-soluble A which she ingests in her large intake of leaf and seed products, and thus enable the young to obtain a much larger amount of this substance than it could possibly obtain by eating the food taken by the mother, because of the limited capacity of its digestive apparatus. The same is doubtless true of the second dietary essential of unknown chemical nature, water-soluble B, but this would seem not to be a matter of great significance since this substance is so abundant in most of the natural foods that the young could easily secure enough for its needs in the limited amounts of food which it could ingest.

Milk represents a very concentrated food from the standpoint of energy and protein values (although associated with much water), is easy of digestibility and is, when drawn by sucking, nearly free from bacteria. Milk is therefore a food, the elaboration of which involves the functioning of the mother as a protective agent in her relation to her offspring. There are further to be considered certain possible synthetic powers which the mammary tissue may possess, in which it may surpass the powers of the tissues of the growing young.

Milk sugar, the only carbohydrate of milk, is found nowhere except in the secretion of the mammary gland. It affords an example, therefore, of a special synthesis by the maternal organism for the nutrition of the young. Like examples of the exercise of a synthetic function by the mammary tissue in milk production are seen in the peculiar nature of the fatty acids of low molecular weight found in milk fats. These acids do not need to occur in the food of the lactating animal.

The fact that the mammary gland is able to synthesize the sugar lactose and certain of the fatty acids which are peculiar to the milk fats is suggestive of the possibility that these tissues may be able to effect the transformation of certain amino-acids into others in a manner not possible to the non-lactating animal or to the growing young. There is recorded in the literature an experiment by Osborne and Mendel (26) which was interpreted at the time it was carried out as constituting a demonstration of the ability of the mammary gland to synthesize the diamino-acid lysine, and as supporting the idea that certain transformations of amino-acids not possible to the immature tissues of the young may be effected by certain tissues under special conditions, such as for example in milk formation.

Owing to the fact that certain unwarranted assumptions were made at the time their experimental work was reported, there is much misconception as to the special rôle of the amino-acid lysine in nutrition. They employed diets which contained 28 per cent of "protein-free milk" and added gliadin as the sole purified protein. Osborne and Mendel (27) assumed that the gliadin employed "does not yield more than insignificant amounts of lysine" (p. 342), and interpreted their data as showing that "certain proteins, notably the gliadin of wheat, may supply the nitrogenous needs of an animal in maintenance, yet be entirely inadequate for the purposes of growth" (p. 328). They further state (p. 332), "we have succeeded in promoting growth at a normal rate when a maintenance ration containing gliadin as the sole protein was supplemented with lysine," and (p. 333) "the demonstration that the addition of lysine to the gliadin food serves to render this protein of wheat entirely adequate for the nitrogenous needs of growth is shown in chart 1, rat 1113, in the appendix, in which the surprising effect of this amino-acid addition is in strong contrast with the mere maintenance effect of the diet without the lysine," and further, "we believe that these feeding trials, in conjunction with our demonstration of the almost complete cessation of growth on diets containing only lysine-free proteins, furnish the first and only conclusive demonstration that lysine is indispensable for the functions of growth." On page 334, they further state "the animal organism apparently cannot synthesize lysine, which is evidently not essential for maintenance in the sense of preservation of body weight, though it is, of course, impossible to say that when this amino-acid is missing, all functions are normally carried out."

These conclusions, we feel confident, are based upon unwarranted assumptions concerning the character of the food mixtures which these authors employed in their experimental work. It is desirable that so important a deduction as the differentiation between the requirements of the animal for maintenance as contrasted with growth, if unwarranted, should be clearly shown to be fallacious. We take this occasion, therefore, to offer a critique of certain of the conclusions of Osborne and Mendel, which are based upon experiments of such a character as to appear open to criticism only to those who, like ourselves, have studied closely the problems relating to the behavior of animals fed upon diets consisting of isolated and carefully purified foodstuffs, and simplified as far as is possible, consistent with the normal nutrition of an animal.

The quotations above refer to the relation of the amino-acid lysine to the nutrition of the growing young animal. Osborne and Mendel employed a diet of similar composition to that discussed above in its relation to maintenance as contrasted with growth, and describe what they interpreted to be a pregnancy and successful lactation period in a rat (26). Four young were brought to the age of twenty-three days during which they grew at approximately the normal rate, while the mother was restricted to the "gliadin food," which was assumed to contain but an insignificant amount of lysine but to be otherwise complete as a source of amino-acids. They believed that lysine was not necessary for the long continued maintenance of an animal (27), but indispensable for growth, and the conclusion seemed warranted that the mother, taking supposedly lysine-free food and producing during gestation four young, and during lactation milk which was capable of inducing nearly normal growth in the young, was able through the special powers possessed by the mammary tissues to synthesize the amino-acid lysine for the formation of normal milk proteins. Casein and lactalbumen both contain 7 to 8 per cent of lysine.

The "gliadin food" in the lactation experiments consisted of carefully purified gliadin, 18 per cent; "protein-free milk," 28 per cent; starch, lard and agar-agar (26). Gliadin has been shown in a reinvestigation by Osborne to have been erroneously assumed to be lysine-free. Furthermore, for reasons explained later, we are convinced that the dietary properties of "protein-free milk" were not fully understood by these authors. They believed at that time that the gliadin was practically free from the amino-acid lysine and they minimized the possible importance of the nitrogenous components of the "protein-free milk" as a source of amino-acids and drew the conclusion that the mother was effecting a synthesis of this particular protein cleavage product (lysine), since the young were unable to grow on the diet of the mother after the period when they may safely be weaned. Owing to the important deductions drawn from these experiments, certain erroneous assumptions made regarding the quality of the diet used in the experimental work referred to, should be pointed out. Other experiments were reported in the same paper in which steady loss of weight followed when the diet consisted of starch, sucrose, lard, gliadin, salts and agar-agar, and such losses were regained when "protein-free milk" or feces were supplied. This result may have been due to the addition of both the dietary essentials, fat-soluble A and water-soluble B, which were lacking in the purified diet and whose significance was not at that time appreciated, as well as possibly to the supplementary value of the nitrogenous compounds, e.g., lysine, of the "protein-free milk" or feces.

The later discovery by Osborne, Van Slyke, Leavenworth and Vinograd (28) that there is about 1.34 per cent of lysine in the most carefully purified gliadin, and the lack of evidence that "protein-free milk" does not supply lysine to some extent, renders very problematical the correctness of the conclusions concerning the ability of the maternal organism to synthesize lysine through the medium of the mammary gland for the maintenance of the species, as contrasted with the inability of the young animal to effect the same synthesis for its own preservation during growth. The food of the mother contained lysine and the amount of this amino-acid available for the synthesis of milk proteins depended upon the capacity of the mother to consume and digest food protein, poor in this complex, above her own body needs.

"Protein-free milk" contains about 0.76 per cent of nitrogen and a diet containing 28 per cent of this substance derives 0.2128 gram of nitrogen per 100 grams of ration from this source. When a food mixture is prepared, as were many of those employed by Osborne and Mendel, by the combination of 18 per cent of purified protein with 28 per cent of "protein-free milk," and the remainder of the food mixture was composed of nitrogen-free substances, the resulting food mixture derives 93 per cent of its total nitrogen from purified protein and 7 per cent from the "protein-free milk." The proportion of the total nitrogen of the diet which comes from the uncharacterized forms in "protein-free milk" rises, of course, when the amount of purified protein is decreased. In some of their experiments in which the purified proteins were fed as low as 2 per cent of the food mixture, the assumption was made that this purified protein furnished the sole significant nitrogen from the standpoint of nutrition, but in reality 63 per cent of the total nitrogen of the diet was derived from the "protein-free milk" (29). McCollum and Davis have presented evidence which indicates that this is a source of amino-acids (30). This so-called "non-protein" nitrogen has been consistently ignored as being of no biological value, but that Osborne and Mendel now appreciate its significance is shown by a recent publication (31).

Munk (32) stated that about $\frac{1}{16}$ of the total nitrogen of milk is in the form of "non-protein" nitrogen. By this he meant that it could not be precipitated by such reagents as alcohol, tannin, copper hydroxide, etc. Osborne and Mendel state, "since our protein-free milk powder was equal to 50 per cent of the total solids of the milk, it should, *if Munk's statements are correct,*[4] contain 0.48 per cent of non-protein nitrogen, thus leaving at the most only 0.28 per cent of protein nitrogen equal to 1.69 per cent of protein. Since 100 grams of the food mixture employed in our experiments contained 28.2 grams of protein-free milk powder, *we can assume*[4] that at most the food pastes thus made contained only 0.48 per cent of milk protein" (33). They further stated in commenting upon the composition of their diet of gliadin, "protein-free milk," starch, lard and agar-agar: "such analyses as we have made indicated that the extent of this contamination (with protein other than gliadin)[5] cannot exceed 0.6 per cent of the entire food mixture, a quantity of "normal protein" far too small as, we have convinced ourselves by other studies directed to this point, to meet the nutritive deficiency of gliadin in respect to growth (26).

All ordinary milk is infected with bacteria during the process of milking, and it is well known that milk contains proteolytic ferments which may well within a few hours convert a significant amount of the protein of the milk into cleavage products sufficiently simple to escape precipitation by the protein precipitants. Milk is also known to contain all the constituents of the blood in small amounts and modern researches of Folin and Denis (34) and Van Slyke and Meyer (35) have demonstrated that the blood is a dilute solution of amino-acids. All things considered we believe that the assumption is unwarranted that "protein-free milk" may not serve as a very significant source of various amino-acids. In the experiments described the assumption was made that the lactating mother was constructing from gliadin, supposed at that time to be practically free from lysine, the milk proteins which contain an abundance of this cleavage product of proteins. Accordingly, the assumption that growth of the young which were nursing the mother on this relatively lysine-poor diet affords proof of the synthesis of lysine or a difference between the chemical requirements of an animal for growth as contrasted with maintenance, cannot be regarded as resting upon a sound experimental basis.

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[4] Italics, ours.

[5] Words in parentheses, ours.

The considerations which have been discussed in their relation to the validity of the proof of the ability of the mammary gland to produce syntheses not possible for the other tissues of the body, and the theory that the processes of maintenance differ chemically from those of growth, serve likewise to emphasize further the fact that feeding experiments in which "protein-free milk" is used cannot be regarded as in any way comparable with experiments in which the diets consist of carefully purified food substances, together with butter fat as a source of fat-soluble A and suitably prepared extracts of natural foods to furnish the second unidentified dietary essential, water-soluble B. Only with diets of this type can the individual proteins be compared in a way which reveals their relative biological values. "Protein-free milk" contains a liberal amount of water-soluble B, and a small and inadequate amount of fat-soluble A (31). Much of the work done with diets containing "protein-free milk" may possibly have led to correct conclusions, but it is impossible to tell which of the results are trustworthy and which are not until the work is repeated with suitably controlled diets.

Hart and Humphrey (36) have reported the results of several experiments so planned as to compare the efficiency of a number of protein mixtures for transformation into milk. These show decided differences in the biological values of proteins for milk production, entirely analogous to what is well established with respect to difference in the values of individual proteins for growth (37).

As yet it has not been shown that the mammary gland is any more efficient in the utilization of food protein for milk production than are the tissues of the young of a rapidly growing species for the construction of new body tissue. Apparently the same limitations hold in both cases.

The quantitative comparison of the ability of the young to utilize food proteins for growth as compared with the ability of the mammary gland to utilize them for the synthesis of milk proteins, is attended with peculiar difficulties. The rate at which the young can be expected to utilize protein for storage as new body tissue will be determined by the "growth impulse" of the species (37). The human infant is not capable of multiplying its initial weight by much more than 3 during the first year of life and cannot accordingly be expected to retain a high percentage of the protein taken as food for the formation of body tissue. Its growth impulse is low. The domestic pig, on the other hand, has the greatest growth impulse of any species with which we are familiar. It is capable, when its diet is highly satisfactory, of multiplying its initial weight by about 200 during the first year of life. The pig is capable of retaining protein taken in the food for the formation of body tissue, at a much greater rate than is any species whose capacity for growth is decidedly less than its own. It is only by making use of the most rapidly growing species of which we know that we can expect to secure data as to the actual biological values of a series of proteins. Only the most rapidly growing species can be expected to retain food protein at the maximum rate made possible by the peculiar relationships among the amino-acids which it yields on digestion.

A similar difficulty is met with in attempting to determine the value of the food proteins for the formation of milk proteins. The extent to which the lactating animal is capable of converting food into milk depends not alone upon the quantity of the food, her capacity to digest and assimilate food and on the quality of the food proteins, etc., but likewise in no small degree upon the inherited milk-producing tendency of the individual. The results of the extensive testing of individual cows by the Babcock fat test during the last twenty-five years, have revealed the fact that in most dairy herds there are some cows which are not capable of profitable production, even when given the best food and care. Milk-producing capacity is an inherited trait and it is only in animals with the power of lactation highly developed that one may expect to observe utilization of food proteins for transformation into milk proteins at the maximum rate at which the quality of the food proteins, depending upon their yields of the essential amino-acids, will permit.

Variability in the composition of the milk depending upon the composition of the mother's food. A number of investigations have been directed toward the study of the variability in the content and relationships among the inorganic elements of the milk as influenced by the character of the food. Von Wendt (38) found that the extent of the variability of any of the constituents of the milk is very slight, too slight indeed to be considered as of significance as regar[illegible] the nutritive value of the secretion. In his experiments cows were fed mixed rations of fairly satisfactory quality for milk production. To these he added certain salts and studied the possibility of *increasing* the output of any element in milk. It is not surprising that he was unable to do this since the kidneys and intestine aid in preventing an accumulation of any mineral elements in the blood.

G. Von Bunge states that as early as 1872 he called attention to the question as to whether the mammary gland secretes inorganic constituents in the same relationship as they occur in the ash of the suckling (39). He called attention to the fact that the blood has an entirely different inorganic content from that of the milk which is secreted, although the mammary gland extracts the inorganic materials from the blood stream which flows through it. The following table has become a classic as an illustration of the relationship between the character of the inorganic content of milk from different species of animals and the content of the same elements in the young animal.

100 PARTS OF ASH CONTAIN	NURSING YOUNG ANIMAL			DOG MILK	DOG BLOOD	DOG BLOOD SERUM
	Rabbit	Dog	Cat			
K_2O	10.8	8.5	10.1	10.7	3.1	2.4
Na_2O	6.0	8.2	8.3	6.1	45.6	52.1
CaO	35.0	35.8	34.1	34.4	0.9	2.1
MgO	2.2	1.6	1.5	1.5	0.4	0.5
Fe_2O_3	0.23	0.34	0.24	0.14	9.4	0.12
P_2O_5	41.9	38.8	40.2	37.5	13.2	5.9
Cl	4.9	7.3	7.1	12.4	35.6	47.6

Bunge pointed out that the fact that the potassium content of milk ash is somewhat greater than in the ash of the suckling is to be accounted for on teleological grounds. The growing animal is relatively richer in potassium than in sodium. This depends on the increase in the content of the potassium-rich muscle and the relative decrease in the proportion of sodium-rich cartilage.

The great and constant differences in the ash of the blood and milk are sufficient to dispose of the argument that the secreting gland acts through the principle of filtration, removing the proteins, etc., from the blood.

Babcock (40) has described experiments with cows which he deprived of salt (NaCl) during lactation, and his results are of special interest in relation to the interpretation of our data. He deprived cows of sodium chloride, other than that contained in their food, for varying periods up to fifteen months and noted no decrease in the yields of milk up to a short time before the animals began to fail rapidly. Some actually died from sodium chloride starvation and others were saved from death by the administration of salt. The fat content of the milk of cows receiving an inadequate salt supply was slightly higher than in milk from the control group.

Interesting observations showing the influence of over-feeding of sodium chloride on the character of the milk of cows are also cited by Professor Babcock (40). Lowe in 1861 found 13 per cent of solids in the milk of a cow receiving 2.5 ounces of salt per day. She was given double this amount for three days and the milk on the fourth day contained but 8 per cent of solids. After this the cow was given the usual 2.5 ounces of salt per day and the milk gradually returned to its normal composition. Mendel (41) reports an experiment in Switzerland which showed marked decrease in the solids of the milk as the result of feeding high intakes of salt to dairy cows. This effect is, of course, due to an additional excretion of salt in the milk and with an excessive output of water, which dilutes the milk.

The effects of under-feeding and of faulty diets on the persistence of milk secretion in the lactating female. Eckles and Palmer (42) have conducted very thorough and painstaking experiments on the influence of under-feeding on the flow and composition of the milk in cows. Their results reveal the fact that in the early part of the lactation period, cows are able to remain stationary in milk flow and produce the normal amount for forty days with only 60 to 75 per cent enough energy after subtracting the maintenance requirement. They observed a marked difference in the effect of a subnormal plane of nutrition on the milk flow depending on the part of the lactation period in which the experiment was carried out. During the later portion of the lactation period there is a reduction of the flow in response to under-feeding while this, as stated above, is not the case when the under-feeding follows closely upon parturition. Eckles and Palmer explain this difference on the assumption that milk production is caused by both a chemical stimulus and a nervous stimulus. The former of these is principally responsible for the activity of the mammary gland during early lactation and decreases in its intensity as the lactation progresses, and is finally replaced in great measure by the nervous stimulus. So long as the chemical factor is chiefly the regulating factor governing the gland, milk production is in great measure independent of the plane of nutrition of the cow.

If this law holds good generally for lactating animals, the assumption would be valid that in all the nursing experiments reported in this paper in which mother rats were suckling young while taking diets on which the young themselves were not able to grow after the weaning period, the mothers were producing approximately the normal amount of milk.

In the charts are shown records in which young rats grew at half normal rate on the milk of mothers whose food was of such a nature that it could not produce growth directly. We have the analogy with the cow as evidence that they were producing approximately the normal amount of milk, and it would seem to be the only logical conclusion that the young failed to grow at the full normal rate because of the poor quality of the milk produced.

If then, as seems to be demonstrated by the available data, the mammary gland has no special synthetic power in the synthesis of amino-acids and is limited in the same way as is the young to a supply of these in the food, and as we have previously shown, the dietary essentials fat-soluble A and water-soluble B are not found in the milk unless they are in the food of the mother (43), it becomes of special interest to discover in just what manner the mother acts in a protective capacity toward her young when her own diet is itself inadequate for the nutrition of the young.

The effect produced in the young rats suckled by mothers whose food supply was limited to a single grain, as wheat, maize or oat kernel, with or without single or multiple additions of purified food substances was, it seems certain, the result of the poor quality of the milk rather than of a limitation in the supply. A fact not hitherto appreciated if indeed at all suspected, viz., that the quality of milk as judged by its growth-promoting power is dependent in a surprising degree upon the quality of the diet of the mother, is shown in a conclusive manner. The differences in quality, if we can judge by analogy with the cow, are of such a nature as not to be detectable by any of the ordinary methods of chemical analysis. Even extraordinary methods of chemical analysis could reveal the deficiencies of such milk only with respect to its inorganic content.

We have sought in the experiments reported in this paper to analyze by the biological methods, which we have made use of in the study of the problems involved in the nutrition of the growing young, the peculiar rôle of the mother in safeguarding the young in early life.

These experiments are not directly comparable with those of Eckles and Palmer in certain important respects. They fed mixtures of leafy foods and grains, mixtures which we have shown to be dietetically complete (44) in that all the essential dietary factors were present and in no very great degree were there unsatisfactory proportions among the several factors. Their experiments involved only limitations as to amount. Our experimental conditions reported in this paper imposed special features—dietary problems not met with in the case of the herbivora but common enough among human beings who do not make use of dairy products as foods, unless they partake of the leafy vegetables in much greater amounts than are commonly eaten in this country. Eckles and Palmer were feeding a diet which would induce growth in a young animal if supplied in sufficient amount. The seeds which formed the basis of our food mixtures would not support growth because of three types of deficiencies, viz., inorganic content, protein and fat-soluble A. With such diets the distinct limitations of the maternal organism in the matter of secreting normal milk are easily seen.

The results reported with cows are in harmony with those reported by Decaisne (45) who observed that during the siege of Paris in 1870, young and vigorous women maintained without loss of weight and in some cases induced gains in their infants, when they were themselves fasting or nearly so.

The studies here reported (charts 1 to 6) show that the lactating mother is limited in a general way in all respects as are the growing young with respect to her ability to effect chemical transformations of one food complex into another, utilizing food proteins for milk production only to the extent that they yield amino-acids in proportions suitable for rearrangement into milk proteins. She is, however, a very important factor of safety for her young in that her mammary tissues can remove from the blood all elements necessary for the production of milk, approximating more nearly the normal in quality than was the food from which it was produced. She can pass these on into the milk in decidedly more favorable relationships than they exist in her food.

This the mammary gland can do when nourished by blood which contains certain inorganic elements in such relationships as render the circulatory fluids of the body a pabulum from which the tissues of the young cannot secure satisfactory supplies to permit the cells to grow, even though the organic portion of the diet is satisfactory. In other words she can present to her dependent offspring a more satisfactory inorganic food supply than she herself receives, and through the medium of the mother the young is enabled to progress well toward the attainment of the adult size in an environment in which the food supply is of such character as would not support growth if it were not for the medium of the mother. (See charts 3 to 5.)

The well established facts concerning the relation between the character of the diet and the formation of milk may be briefly summarized as follows:

1. The evidence seems conclusive that there is no special synthetic power of the mammary tissue by reason of which amino-acids can be formed which are not found in the food. In other words, the maternal organism is limited in milk secretion in the same way as is the growing animal in regard to its amino-acid supply. Both must have the same list of amino-acids preformed and circulating in the body fluids. Diets employed in this type of study must be rigidly controlled so as to preclude the possibility of the animals procuring the special complexes from "protein-free milk," feces, etc., on whose absence the validity of the observations rest.

2. The mammary gland performs the function of elaborating proteins of extraordinary biological value from the products of digestion of the food proteins. This involves no synthetic activity by which one amino-acid is transformed into another but only a selective absorption from the blood and reconstitution of the amino-acids which circulate in the blood into complexes, the milk proteins, which in turn are, when taken as food, very efficiently transformed into tissue proteins for growth.

3. The mammary gland picks up from the blood both the chemically unidentified food essentials, fat-soluble A and water-soluble B, and passes these into the milk, but it is unable to produce either of these substances anew. When one or the other of them is absent from the mother's diet, it is not found in the milk (43). We have shown the possibility of producing milk, poor or lacking in each of these substances and therefore not capable of inducing growth.

4. The mammary gland has the capacity to function approximately

normally when nourished by a blood stream which contains the mineral elements, sodium, chlorine and calcium in amounts too low to enable the tissue cells to grow. This is shown by the fact that the inorganic content of the milk produced from a diet whose content of these elements is too low to admit of growth in the young is of distinctly better quality for supporting growth than is the food from which it was formed. This represents a very important factor of safety in the preservation of the species.

5. It is evident from the character of the curves of growth of the young in charts 1 to 6 that the quality as well as the quantity of the protein of the diet, together with the content and character of the inorganic portion of the food supply, are factors of importance for milk production commensurate with that of an adequate amount of both the fat-soluble A and water-soluble B.

6. Even after the young has attained a stage of development at which it can eat of the mother's ration, the supplementary contribution of milk by the mother serves to greatly enhance the proteins derived from vegetable foods and to partially correct the inorganic faults in the latter as well as to give the young a much greater supply of fat-soluble A than it could itself ingest in the form of vegetable foods. Fat-soluble A is not abundant in the vegetable foods and the action especially of the herbivorous mother in concentrating in the milk fats the intake of this substance in her large intake of food, constitutes a surprising protective relationship of the mother to her young. The results of the present series of experiments show that milk is superior to the vegetable mixtures on which the herbivorous mother feeds in its physical properties, its easy digestibility and in its favorable inorganic content.

The experiments described in the charts demonstrate the following facts: (1) That the mother may take a single seed as food and produce milk in sufficient quantity and of such quality that growth can proceed at a subnormal rate whereas the young could not themselves grow at all on the mother's diet; (2) when the inorganic deficiencies of the seed are corrected, the mother can induce much better growth in the young than without such correction; (3) *the improvement of the protein or the fat-soluble A content of the seed causes little improvement in the quality of the milk when the mineral content of the diet remains uncorrected.*

These results for milk production are in agreement, except in degree, with the behavior of young when fed a seed supplemented in the same manner. The mother can take a seed as her sole food and put into the milk an inorganic salt content which is very much more satisfactory for the nutrition of the young than is that contained in the seed. It is in this respect that she serves as the greatest factor of safety in the nutrition of her young. The other ways in which the maternal organism assists the relatively helpless young in its nutrition have been already pointed out.

It is now well established that the seeds, tubers and root foods are closely comparable from the dietary standpoint in that their deficiencies are of the same type and order, all being storage organs relatively poor in cellular elements. Muscle tissue is principally composed of highly specialized contractile tissue and contains but relatively little substance comparable in its metabolic activity with the cellular organs such as the liver, kidney and other glands. It should, according to our theory of the relation of dietary properties to function, be classed with the storage organs of plants rather than with cell-rich tissues such as the leaf. It is not possible to make up diets derived from even these four types of foodstuffs (seeds, tubers, roots and muscle tissues (17)) in any combinations which will induce normal nutrition. It should follow from the studies now reported, and there can be little doubt of the correctness of this view, that milk produced by a mother whose food consists entirely of seed products, tubers and meat, will not be of very satisfactory quality for inducing growth.

DISCUSSION OF RESULTS

We have, in previous papers, shown that there is but one way in which an adequate diet can be made up entirely from vegetable sources, provided we ignore the possibility of the chance supply through the drinking water of such a mineral element addition to the diet as will make good the deficiencies of all the seeds in calcium, sodium and chlorine, viz., by making suitable combinations of the leaf and seed (44). There is small chance that a diet derived entirely of seeds will, even when the water furnished contains the proper minerals, prove of very satisfactory quality for the support of growth. We have emphasized the fact that the present tendency in parts of the United States to derive the diet almost entirely from seeds, tubers and meat, is responsible for the lowered vitality, loss of efficiency and the appearance of diseases of dietary origin (46). Our studies also afford abundant proof that highly successful diets can be made up of mixtures of seeds and milk (17). The importance of the dairy industry as a factor of safety in the nutrition of the adult population of the more progressive nations of the world makes the results of these studies of the relation of the mother to the young applicable to the every day problems in the nutrition of man, adult as well as infant.

The three most important seeds employed as foods in America are the wheat, oat and maize kernels. Each of these contains all the elements and chemical complexes which are essential in the diet of the growing young animals, yet each when it serves as the sole source of nutriment for a young animal fails to induce any growth and cannot long maintain life. The most elaborate chemical analysis throws practically no light on the reasons for the failure of nutrition in animals so fed. Even mixtures of these three seeds, in any proportions, do not support growth. The reasons for this we have shown by our biological method for the analysis of foodstuffs (22) to rest in the inadequate character of the inorganic moiety of the several grains as the first limiting factor, the shortage of the fat-soluble A as the second, and the relatively poor quality of the proteins as the third factor. The latter fault is less pronounced in mixtures of the grains than in each of them as the sole source of protein.

Among peoples who subsist almost entirely upon polished rice and fish, the disease beriberi is of common occurrence and it not infrequently happens that infants nursing mothers suffering from the disease likewise contract beriberi in the first months of life. The cause of beriberi is now well known to be the consumption of a diet which may be unsatisfactory in several respects, but the specific cause of the syndrome is the selective fasting of the individual for the unidentified dietary essential water-soluble B (4). Diets so made up that they are known to be adequate in every respect, except for the shortage of this substance, induce the onset of the disease within a few weeks. Andrews (47) attempted to throw light on the etiology of infantile beriberi by inducing Philippine women, whose infants had just died of beriberi, to nurse young pups. These in all cases developed the paralysis of the posterior extremities which is one of the characteristic symptoms of the disease. The cause of the disease had not at that time been established, whether it is due to some toxic agent or to a lack of some essential substance in the diet. Andrews' experiments are of great interest in connection with our own, in which we made up diets which were deficient only in respect to the dietary factor, water-soluble B, and observed that young rats were unable to grow on the milk of mothers after the latter were restricted for a few days to these food mixtures, but responded with growth in about forty-eight hours after the addition of an alcoholic extract of wheat germ, which supplied the missing dietary essential (43). Andrews' studies with human milk are confirmed and explained by our own with rats, and establish the fact *that milk production may take place under dietary conditions such that the product is deficient in certain complexes necessary for growth.*

We have recently called attention to the fact that there is a second dietary deficiency disease analogous with beriberi, in that it is the specific result of a shortage of the chemical substance fat-soluble A in the diet. This is a type of xerophthalmia of dietary origin and is characterized by an edematous condition of the eyes, followed speedily by blindness unless the missing dietary essential is supplied (5). Clinical evidence is available in the observations of Mori (48), Bloch (49), H. Rønne (50), Czerny and Keller (51), that this syndrome which became known through nutrition studies on animals, has repeatedly occurred as a human disease.

As we have elsewhere pointed out (5), there is but one way to produce this condition of the eyes, viz., by selectively fasting an animal for the dietary essential fat-soluble A, and it can be relieved only by supplying in the diet the missing dietary complex. From among the numerous citations in the literature of eye affections among poorly nourished peoples in many parts of the world, we have found it impossible to decide whether the xerophthalmia of dietary origin has been sometimes confused with trachoma or other eye infections. It will be of special interest if clinicians will keep in mind the possibility that edema of the tissue surrounding the eyes, inflammation of the cornea with or without perforation, followed in extreme cases by blindness, may have their origin in a specific lack in the diet in a manner entirely analogous to beriberi. The subject is mentioned here because milk may be very poor in the dietary essential, fat soluble A, when the diet of the mother is derived almost wholly from seeds and seed products, especially those derived from the endosperm, e.g., wheat flour, degerminated corn meal, polished rice, starch, sugars, syrups, together with vegetable fats or the reserve fat deposits of the animal body (lard, suet, etc.).

The light which the experimental data described in this paper throws upon the relation between the quality of the diet and the quality of the milk as a food, may be considered in its possible relation to the high incidence of rickets among infants in classes of people who live in poverty. Among the very poor there is a strong tendency to restrict the diet to bread and other seed products, syrups and molasses, together with potatoes, sweet potatoes and small amounts of meat. We now know that such diets do not produce satisfactory results in the nutrition of animals, and that the milk of the mother taking such diets is likely to be of very poor quality as food for her infant. It seems not improbable that future investigations may show a relationship between the lowered resistance of infants so fed and the development of rickets, whatever may be the exact nature of the immediate causative agent of the disease. Hess (52) has recently described the diets of negro mothers in the Columbus Hill District in New York City, whose infants almost universally suffer from rickets. The mothers' diets consist of seed products, tubers and meat almost exclusively. Milk, the leafy vegetables and fruits are scarcely eaten at all.

One of the most interesting and surprising facts which has come from our systematic studies with simplified diets is the remarkable dependence of the young animal upon a suitable content of several mineral elements in its food supply. An animal cannot grow on one of the cereal grains as the sole food supply but if 1 per cent of sodium chloride and 1.5 per cent of calcium carbonate be added to a cereal grain a considerable amount of growth may take place. All the elements contained in these salts are present in the seeds but not in sufficient amounts. Apparently the kidney fails to hold back these elements as effectively as it should and thereby renders the young animal much more in danger of failing to secure a sufficient intake of these elements than would be the case if the kidney were able to excrete the waste products of metabolism and at the same time hold back and permit to accumulate in the blood stream and lymph a concentration of mineral elements which would be favorable for the nutrition of the body tissues during growth. This it fails to do, and growth can take place in those mammals which have been studied only when certain mineral elements are coming from the alimentary tract at or above a certain rate. The kidney seems, therefore, in some cases involving a low intake of inorganic salts in the diet, to be the tissue whose limitations determine the ability of the young animal to grow.

A suggestive feature of the results of these studies is the deduction which may reasonably be drawn concerning the quality of the blood as a pabulum for the nourishment of the tissues in general when the diet is derived too exclusively from the seeds of plants. It has been pointed out how different is the inorganic content of the milk as compared with that of the blood serum, and it is well known that the proteins of milk are the specific product of the mammary tissue and are not found in the blood.

The lactose is derived from the glucose which is ever present in the blood. These facts seem to indicate the inability of the mammary glands to function properly because of the poor quality of the blood in respect to salts or protein cleavage products, or to the fat-soluble A or water-soluble B, when the blood gets its supply of these substances from an intestine which is digesting cereal grains exclusively. As stated above, the product of the mammary gland is specific and widely different from the blood, yet it is sensitive to such changes in the blood as may arise from the too exclusive eating of seeds. The question which suggests itself is, how do the remaining tissues of the body fare when purveyed to by a blood which does not admit of the production of growth-promoting milk? Diets derived almost exclusively from seeds such as peas, navy beans, corn, wheat, rice and rolled oats and tubers, such as potato, cause stunting and admit the early onset of signs of senility, and the span of life is short (17). The debilitating influence of such diets is a factor in the public health which should be more generally appreciated than it is both by the physician and the laity. It requires grave dietary faults to produce such serious conditions as scurvy, rickets and beriberi. There are tens of thousands of human mothers who are attempting to nurse infants on diets derived too largely from the seeds of plants and their milled products. Such diets produce inferior milks and may be the cause of grave disorders in the very young. Dietary mistakes of a minor character which debilitate the tissues and lead to inefficiency, lack of resistance to bacterial infection, and pave the way to the onset of errors in metabolism are very common. In but few instances does the human machine run past middle life without developing a rattle somewhere. It is clear from experimental evidence now available that there are causes other than those proposed by Metchnikoff responsible for the gradual diminution of vitality which daily brings us nearer to the condition where we are classed with the great army of the unfit.

It is certain that milk is a better food for the young during the suckling period that is any ration which can possibly be compounded from vegetable sources. If the mother is practically limited in respect to the synthetic capacity of her mammary tissue in the same way that the suckling is, except in the formation of milk sugar from glucose, is it only in the physical properties and digestibility of milk and in the peculiar character of its carbohydrate that milk is more suited for the nutrition of the suckling than is the coarser food eaten by the mother? The experiments described in this paper indicate that a shortage of certain inorganic salts in the diet of the lactating mother may become a factor of great importance in determining the quality of the milk. (Compare charts 3, 4, 5 and 6.)

It may be argued that the faulty diets employed in our experiments (charts 1 to 6) caused depression of the growth of the young by reducing the milk production of the mothers rather than by changing the composition of the milk with respect to some one or more of the important constituents. We cannot state from actual records of milk production in the rat whether the volume of milk produced remained sufficient for the growth of the young throughout the periods of depressed growth, but the observations on women (45) and cows (42) just cited bear directly on this point. Furthermore, in our own records young are regularly shown to grow long after they become able to eat of the mother's diet, when the latter is of a character which cannot support growth when it serves as the sole food supply. There can be no other explanation for this than that the mothers continue to supplement the food supply of the young with fairly liberal contributions of milk.

From the experiments just cited we have data strictly comparable to our own where mother rats are limited for their supply of mineral elements to the single seed which furnished the major portion of the diet. The seeds, wheat, oats and maize, we have shown are too poor in sodium, chlorine and calcium to permit young animals to grow appreciably, and we see in the case of the lactating mother attempting to induce growth in a litter of four young an inability to produce milk of a character which enables the young to grow beyond a limited rate, but the milk is better for the nutrition of the young than is the mother's diet. From the data furnished by Babcock (40), by Eckles and Palmer (42) and by Decaisne (45), as well as by our own charts, it seems certain that failure to produce a sufficient quantity of milk was not the cause of partial stunting of the young, but rather too low a content of those mineral elements which the seeds fail to supply in amounts sufficient to meet the needs of a growing animal. These experiments seem to afford evidence that diets containing no more of certain inorganic elements than are furnished by the more important seeds employed as human and animal foods may lead to the production of milk of poor quality as a growth-promoting food.

EXPERIMENTAL METHODS

In order to study the extent to which the mother can serve as a protective agent in producing milk of a character suitable for the nutrition of her young when she is fed upon a diet on which the young, after weaning, are not able to grow, we adopted the following procedure: The female rats were fed our 211[6] ration until they delivered their young. The litter was then in all cases reduced to four young in order that the nutritive undertaking of all the mothers should be comparable and in no case burdensome. The mother was then restricted to the diet described in the chart with her curve, and frequent weighings were made of both mother and young, from which the curves were plotted. The curve of the mother is placed at the bottom of the chart and the curve showing the collective weighings of the four young is placed directly above. A drop in the curve of the young indicates that at that point the litter was reduced by the death of one or more, to three, two, etc., young. Supplementary data regarding the condition of the young and mothers are presented in tabular form in tables 1, 2, and 3.

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[6] Wheat	64.0
Casein	10.0
Skim milk powder	10.0
Salt mixture	3.6
Dextrin	7.4
Butter fat	5.0

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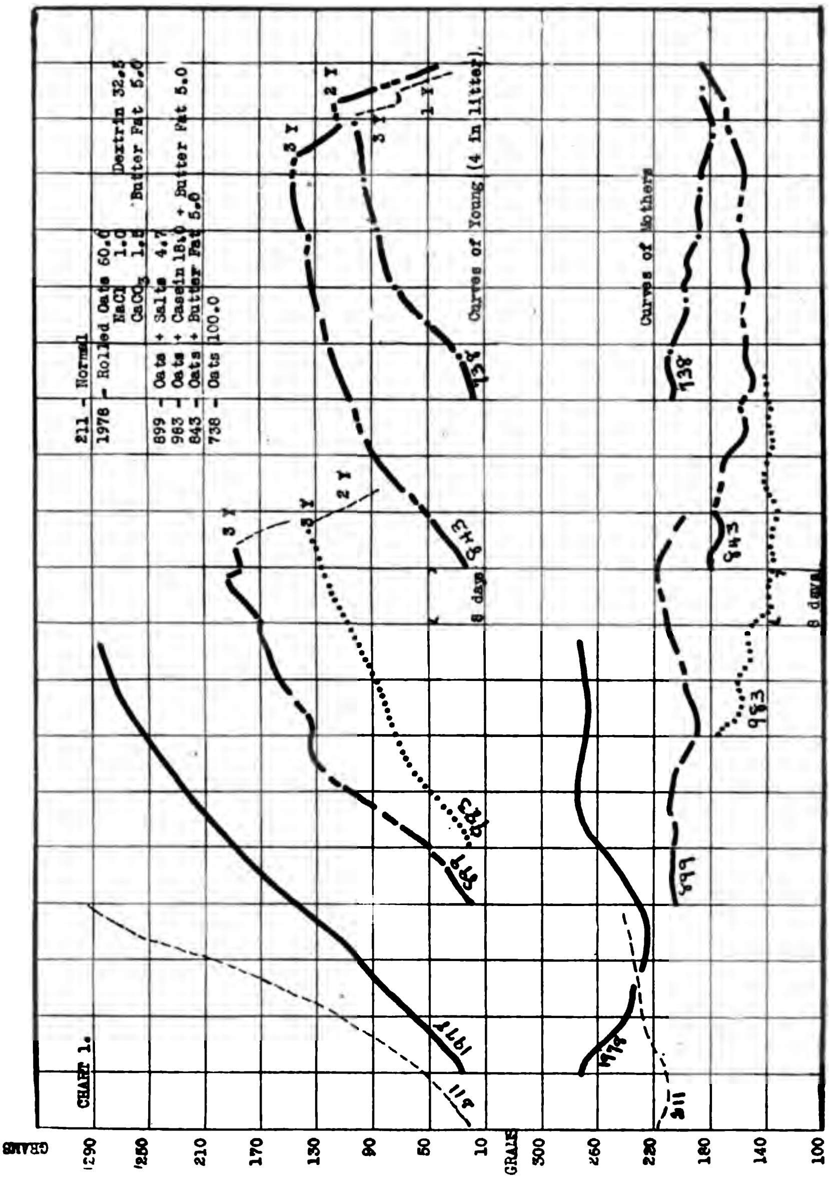
CHART 1.
211 - Normal
1978 - Rolled Oats 60.0 Dextrin 32.5
NaCl 1.0
CaCO3 1.5 Butter Fat 5.0
899 - Oats + Salts 4.7
983 - Oats + Casein 18.0 + Butter Fat 5.0
843 - Oats + Butter Fat 5.0
738 - Oats 100.0
Curves of Young (4 in litter)
Curves of Mothers
3 Y
2 Y
1 Y
8 days
211
1977
1978
899
983
843
738
GRAMS
290
250
210
170
130
90
50
10
GRAMS
300
260
220
180
140
100

Chart 1. These curves illustrate the dietary value of the milk of a mother fed rolled oats alone, rat 738; oats plus fat-soluble A, rat 843; oats plus such salt additions as were necessary to correct the inorganic deficiencies of the oat kernel, rat 899; and oats plus salts plus fat-soluble A, rat 1978. For comparison there is drawn on each chart the excellent growth curve of four young whose mother received our 211 ration, which is one of excellent quality for inducing growth and reproduction (53). The weights represented by the curves are those of the four young collectively.

Rat 738: Diet rolled oats 100 per cent. These young grew decidedly slower than they should up to the 16th day after birth, and increased in weight very little from that time on. They died on the 37th, 40th and 44th days respectively. Rolled oats have been shown (12) to require the addition of three inorganic elements, sodium, chlorine and calcium, an addition of fat-soluble A and an addition of more protein before becoming dietetically complete. The fact that these young grew as much as they did is proof that the inorganic content of the milk was much more satisfactory than that of the oat kernel for the promotion of growth. Rat 899 was able on a diet of rolled oats plus salts to induce much greater growth in her young. The proteins of the oat kernel are of better quality than are those of wheat or maize as is shown by the good growth secured in the young of rat 1978 as compared with lots 1943 (chart 4) and 1942 (chart 6), whose diet consisted of rolled oats plus salts and butter fat (fat-soluble A). The young of rat 738 were, however, not normal for at about the age of 18 to 19 days they were seized with paroxysms in which they would throw themselves about the cage, screaming as if in pain. Within a few minutes they would fall exhausted. This performance they would repeat after intervals of varying lengths. They usually died soon after being observed in this condition.

Rat 843, whose diet consisted of rolled oats and butter fat (fat-soluble A) was not able to induce much better growth in her young than did rat 738 on a diet of oats alone. The first limiting factor in oats as a food for growing animals is the inorganic content of the seed, and the same is true with oats as a food for the lactating mother. The shortage of the factor, fat-soluble A, is also very important for without this being added growth ceased at an early date. The eyes of these young were inflamed but not swollen.

Rat 983 whose diet consisted of rolled oats, purified protein and fat-soluble A, was not able to produce milk of much better quality than she could havedone with either one of these additions omitted. The proteins of the oat do not need much improvement. Although additional fat-soluble A is needed and its addition distinctly improved the quality of the milk as is shown by the fact that growth was continuous at about half the optimum rate during the first 45 days of life, the young at that time began to die. That it was the butter-fat addition which caused such improvement as was seen in the quality of the milk is emphasized by a comparison of the growth curves of the young of rats 738, 843 and 983, chart 1, with that of rat 948, chart 2. In the latter case oats and purified protein (casein) proved to be no better than oats alone for milk production.

Rat 899, on a diet of rolled oats and salts did surprisingly well in inducing growth in her young. Up to the 24th day their growth was nearly normal. At this age young rats begin to eat of the mother's ration. Young rats do not grow at all when restricted to this diet (12). This shows that the mother can

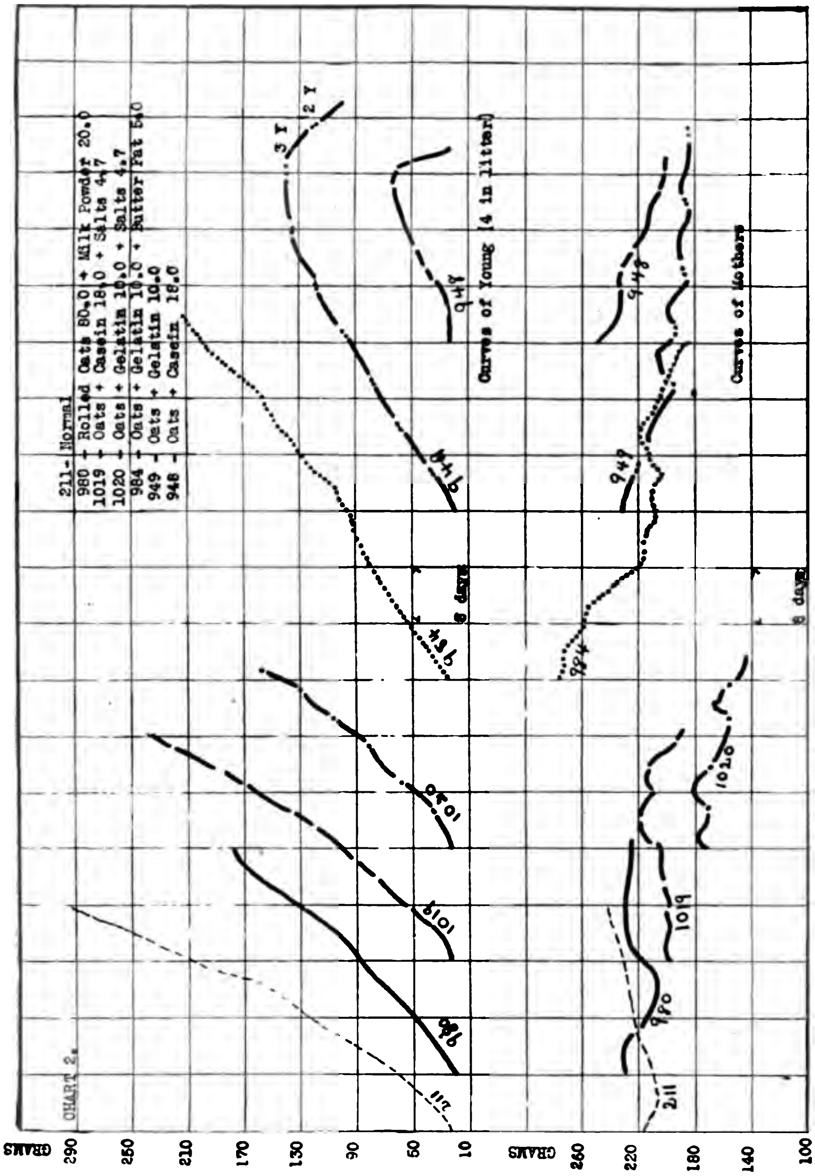
CHART 2.
211- Normal
980 - Rolled Oats 80.0 + Milk Powder 20.0
1019 - Oats + Casein 18.0 + Salts 4.7
1020 - Oats + Gelatin 10.0 + Salts 4.7
984 - Oats + Gelatin 10.0 + Butter Fat 5.0
949 - Oats + Gelatin 10.0
948 - Oats + Casein 18.0
GRAMS
290
250
210
170
130
90
50
10
GRAMS
260
220
180
140
100
8 days
8 days
Curves of Young (4 in litter)
Curves of Mothers
3 Y
2 Y
211
980
1019
1020
984
949
948
211
980
1019
1020
984
949
948

take a diet, the mineral content of which is not suitable for growth in the young, and produce milk which has a distinctly better mineral content for the support of growth than had the food from which she produced it. Before young can grow at all on oats after weaning, both a salt mixture and fat-soluble A must be added. This ability to fare better than other body tissues when nourished by a blood stream which is too low in certain mineral elements to permit of symmetrical body growth, distinguishes the mammary gland from the muscle, nervous and glandular tissues and other tissues of the young. The fact that these young continued to grow between the 24th and 45th day indicates that they were still securing milk from the mother during this period and that this milk supplemented in some degree the deficiencies of the diet of oats and salts.

Rat 1978 whose diet contained but 60 per cent of rolled oats (9 per cent protein) supplemented with salts and fat-soluble A, was able to induce continuous growth at somewhat below the optimum rate over a period of 60 days. On this diet young rats cannot grow to any appreciable extent (12), (54). It follows from this that these young must have been getting such a milk supply from the mother during the entire sixty days as supplemented the oat and salt diet with respect to fat-soluble A and protein otherwise, judging from our earlier records, they should have ceased to grow when the milk supply gave out. It would appear from this that when the female rat is not fertilized she may continue to produce milk for at least sixty days.

Chart 2, completing the series of experiments described in chart 1. Rat 980, whose diet consisted of rolled oats plus 20 per cent of skim milk powder (Merrell-Soule) was able to induce good but not the optimum rate of growth in her young. It is possible that this was due to a low intake of fat-soluble A, rather than to failure of 20 per cent of skim milk powder to supplement the inorganic deficiencies of the oat kernel. This view is supported by the records of rats 1020 and 1019, in both of which oats plus protein and salts were fed and the growth of the young fell below normal because of the inadequate supply of fat-soluble A.

Rats 1020 and 1019 show, respectively, the good growth which the young were able to make on the milk produced by oats supplemented in the first case with gelatin and salts, and in the second by casein and salts. The growth of the young was near the optimum rate in both cases. In a previous paper (54) it has been shown that gelatin supplements the oat proteins better than does casein for the production of growth in the young. From the rate of development of the young on these two rations one would be inclined to pronounce both highly satisfactory, but we know, however, from the results of a long series of feeding experiments, that these diets were too poor in the dietary factor, fat-soluble A to promote optimum wellbeing over a prolonged period. Such studies have made it clear that in order to correctly evaluate any food mixture it is essential that not only "normal" curves of growth be secured, but the animals must be observed until it becomes apparent whether they breed at the same age as do rats on the best rations, and if later, how much. It must be determined whether they produce the normal number of young and rear them almost without exception to the weaning age, and observations should determine whether the second generation can develop satisfactorily on the diet. If the female rat is near the optimum in nutrition, she will become fertilized usually within two weeks after being freed from her nursing young and placed with a male. In ad-

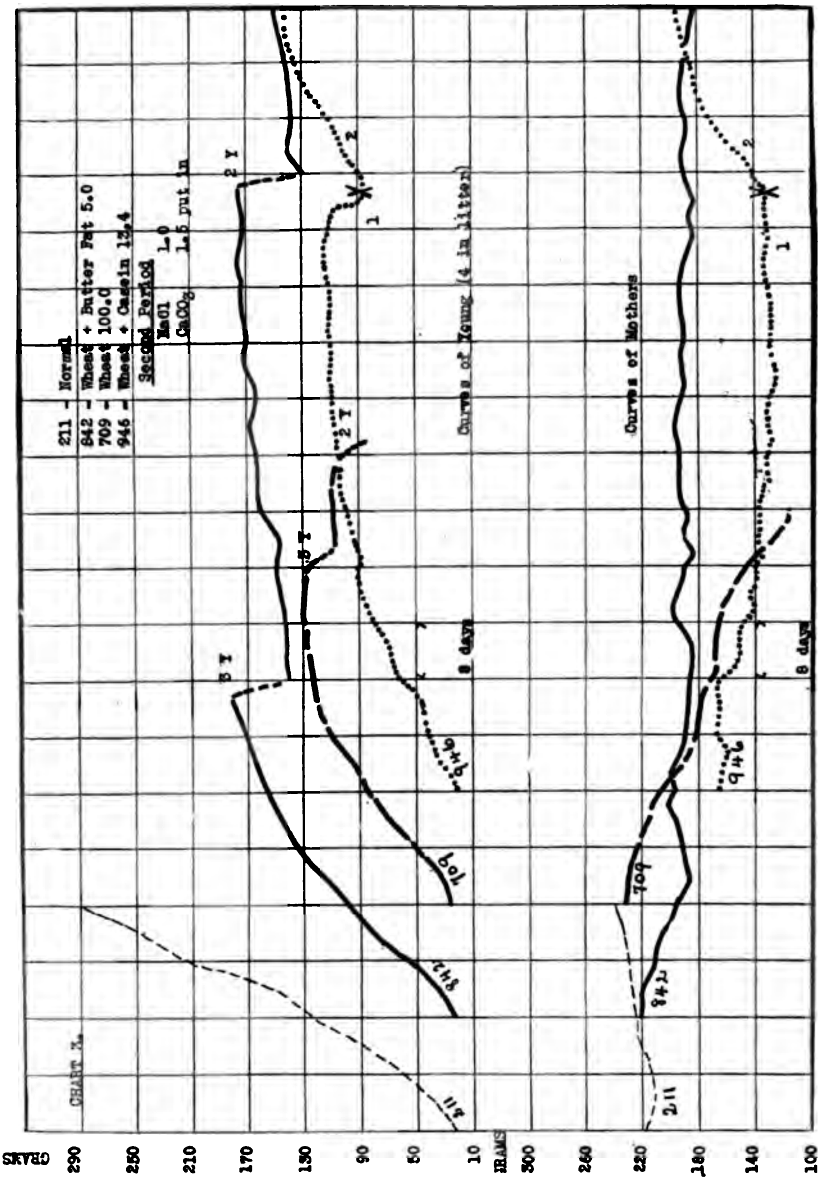
CHART 3.
211 - Normal
842 - Wheat + Butter Fat 5.0
709 - Wheat 100.0
946 - Wheat + Casein 13.4
Second Period
NaCl 1.0
CaCO$_3$ 1.5 put in
Curves of Young (4 in litter)
Curves of Mothers
8 days
8 days
3 Y
3 Y
2 Y
2 Y
1
2
1
2
211
842
709
946
211
842
709
946
GRAMS
290
250
210
170
130
90
50
10
GRAMS
300
260
220
180
140
100

dition to these observations, valuable data can be secured by observing the age at which the animals first show signs of senility as shown by skin and coat changes and by general appearance. *Only with such observations can one say with finality what are the relative biological values of a series of rations.*

The curve of rat 984 presents the records of young which grew at half the normal rate on the milk derived from the mother's diet of rolled oats, gelatin and butter fat. On account of the low content of chlorine, sodium and calcium, this ration will not induce any growth in young animals. The mother was able to accumulate the necessary salts from the blood stream and to secrete a milk which contained a more favorable mineral content than did the food which she was taking. Attention has already been called to similar results with other diets with the same type of deficiency. (Rats 738, 843, 983, chart 1); (rats 949 and 948, chart 2).

Rat 949, whose ration consisted of oats plus gelatin, induced about half normal growth in her young but the young died between the 50th and 60th days. Oat proteins and gelatin constitute a mixture of high biological value for growth (54). The inorganic content of this diet is the first limiting factor as is shown by a comparison of the curves of the young of rat 949 with those of rat 1020. We have not observed good results in feeding growing rats on diets derived principally from rolled oats (12).

Rat 948 received a diet of rolled oats and casein. The result was the production of milk which was faulty in character (if we may safely compare the rat with the cow), and not so constituted as to enable young rats to grow when restricted to it as their sole food. The mortality of the young in the first week of life was 100 per cent for the other six litters which we observed. We have no explanation to offer for this result. (See table 1, chart 2.)

Chart 3. These curves show the dietary value of the milk of the mother rat when limited to whole wheat and to whole wheat supplemented with either purified protein or fat-soluble A. The curve of 211 represents the growth of a litter of four young whose mother was receiving a highly satisfactory diet, described in the legend to chart 1.

Rat 709, which was limited to whole wheat as her sole food, succeeded in producing milk which supported growth in her young during the first 24 days, after which they gained but very little. It is not known how long the young succeeded in extracting any milk from the mother. One young had already died on the 49th day and the remaining ones died on the 53rd and 57th days respectively. We have pointed out that young rats do not grow at all when confined to a diet of whole wheat (10). It follows, therefore, that the mother in this experiment was producing milk from a diet upon which the young were themselves unable to grow and the milk was capable of supporting growth at more than half the normal rate during the first 25 days. Either lactation fell off at this time or the quality of the milk greatly deteriorated as the stores of the mother's body became depleted. The latter explanation seems to harmonize best with the character of the curve of the mother and with the persistence with which other species, as the cow, are known to continue in lactation under conditions of faulty nutrition. On this ration of whole wheat alone, the eyes of both mother and young were inflamed and swollen, indicating partial starvation for the dietary factor fat-soluble A (5).

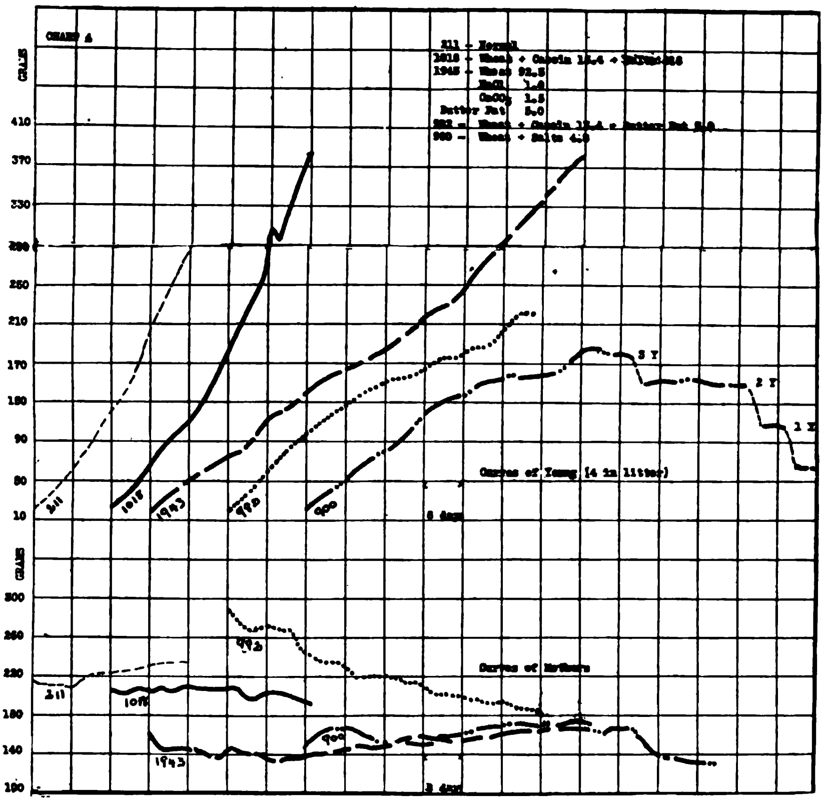
CHART 4
GRAMS
211 - Normal
1018 - Wheat + Casein 13.4 + Salts 4.6
1943 - Wheat 92.5
NaCl 1.0
CaCO3 1.5
Butter Fat 5.0
992 - Wheat + Casein 13.4 + Butter Fat 5.0
900 - Wheat + Salts 4.6
410
370
330
290
250
210
170
130
90
50
10
211
1018
1943
992
900
Curves of Young (4 in litter)
8 days
3 Y
2 Y
1 Y
GRAMS
300
260
220
180
140
100
992
Curves of Mothers
211
1018
1943
900
3 days

Rat 842 received a diet of wheat plus fat-soluble A and induced growth in her young at a nearly normal rate during the first ten days, after which the rate fell off somewhat. They were kept in steady growth during 45 days, however, by the milk of the mother when she was taking a diet which was itself incapable of supporting any growth whatever in the young. The most striking feature of this is in her ability to put into the milk an inorganic mixture of such composition and amount as is greatly superior to that contained in her food.

Rat 946 which was taking a diet of wheat and casein fell slightly below the performance of rat 709, on wheat alone. This is probably to be accounted for by the lowering of the inorganic intake of the mother through the dilution of the wheat with purified casein. The protective capacity of the mother in her relation to her young is easily seen, for on this food mixture young rats cannot grow at all (10) while the lactating mother can take it and produce milk with fair growth-promoting power. Her capacity to do this does not hold up efficiently beyond a limited period, as is to be expected. An addition of a salt mixture was made on the 86th day after the young were born. There was a prompt response with growth in the two remaining young and an increase in weight by the mother.

Chart 4. These records complete the series described in chart 3. The curves of rats 900 and 1943, both indicate that the quality of the wheat proteins is so low that it forms one of the limiting factors in determining the capacity of the lactating mother to produce milk of such a character as to promote growth in the young. The addition to wheat of either salts alone or of salts and fat-soluble A, fails to supplement whole wheat so as to enable her to produce milk of the best quality, as is shown by the shape of the growth curve for the young. Growth was continued in both these experiments far beyond the time at which the young begin to depend in large measure upon the diet of the mother. Since some growth can be secured in young rats fed wheat plus salts alone or with the further addition of butter fat (fat-soluble A) the records here shown do not indicate so great a protective action of the mother in safeguarding the welfare of her young as is seen in certain other cases described.

Rat 982 whose ration consisted of wheat, protein and fat-soluble A was unable to produce milk of excellent quality and there can be little doubt that the limiting factor in determining the quality of the milk was the low content of the elements, sodium, chlorine and calcium in the food mixture of the mother. This is strikingly illustrated by the growth curve of the young of rat 1018, whose ration of whole wheat was improved by the addition of both salts and protein. On this food mixture the mother was able to produce milk of excellent quality.

As just stated, rat 1018 succeeded in rearing young on wheat with protein and salt additions. When her record is considered in comparison with those of rats 900, 982 and 1943, it is seen how dependent the lactating mother is upon the character and amount of the inorganic content of her diet, and the comparison likewise demonstrates that she is much more independent of this factor than is the young rat in its ability to grow.

In the light of these records, the lactating mother stands in a new and hitherto unsuspected relation to diets which are in some degree faulty for the nutrition of the young.

Wheat is shown by these records to be decidedly richer in fat-soluble A, and the behavior of the young to be influenced less by the omission or inclusion of

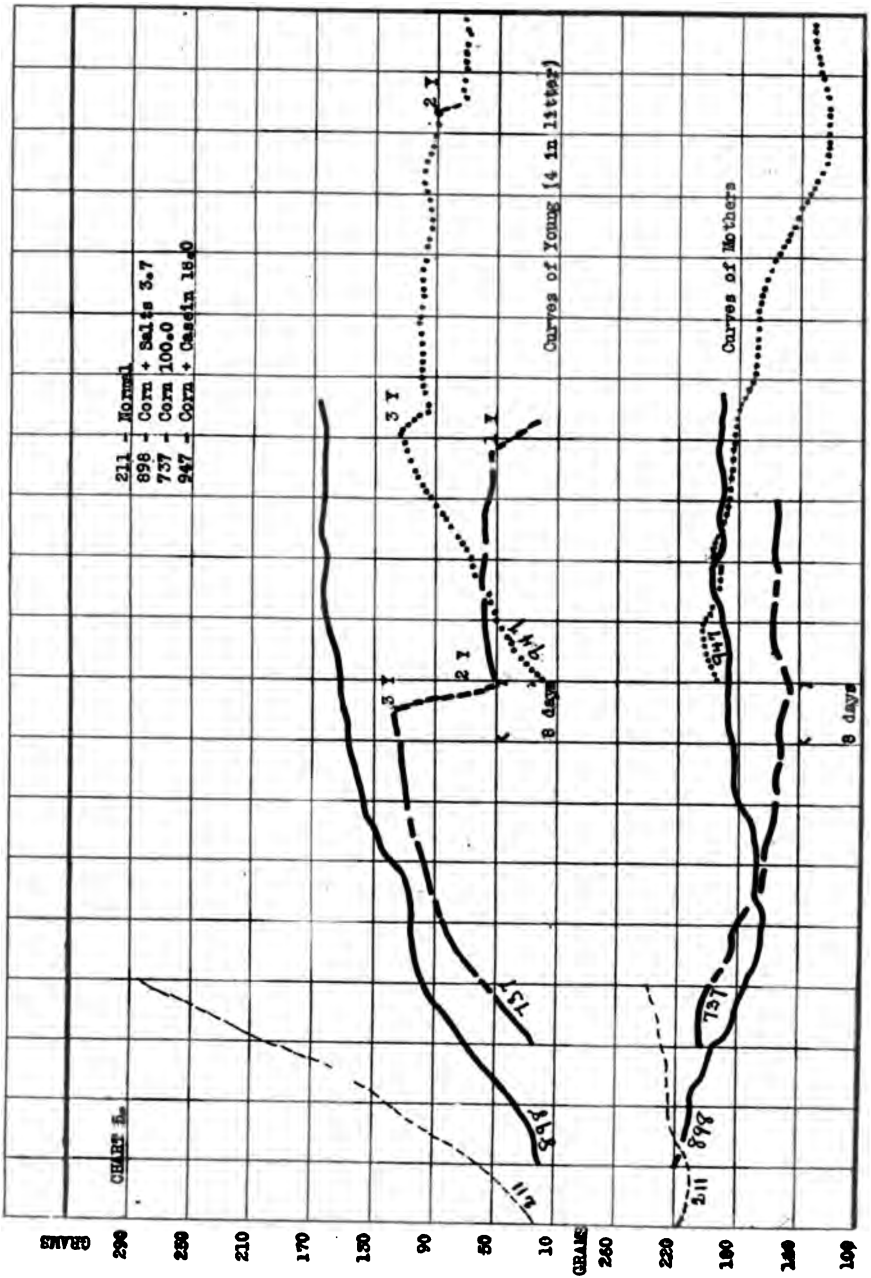

CHART 5.

211 - Normal
898 - Corn + Salts 3.7
737 - Corn 100.0
947 - Corn + Casein 18.0
Curves of Young (4 in litter)
Curves of Mothers
8 days
8 days
3 Y
2 Y
Y
3 Y
2 Y
Y
211
898
737
947
211
898
737
947
GRAMS
290
250
210
170
130
90
50
10
GRAMS
260
220
180
140
100

this factor than was the case with oat diets. (Compare chart 2, lots 1019 to 1020.) The dietary properties of wheat are discussed in full in a former publication (10).

Chart 5. The curves shown in this chart illustrate the behavior of young rats when dependent upon the milk produced by mothers whose diets were restricted to the maize kernel and to maize with single purified food additions.

Rat 737 was restricted to a diet of ground corn. The growth of her young was only about half the normal rate but was continued for three weeks after the young are supposed normally to depend upon the adult diet. This means that the milk which the mother continued to contribute during the entire 45 days of the record served as an important factor of safety for the young. Two young died on the 42d and 45th days respectively, and the others were unable to develop further on a diet of corn plus such milk as the mother was able to contribute after that time.

Rats 898 and 947, whose diets consisted of corn plus salts and corn plus protein respectively, both failed to produce milk which had much growth-promoting power. As in the case of wheat, the two factors of greatest importance in making corn a poor food for milk production are the inorganic content and the poor quality of its proteins. This is made evident by the record of rat 1017, chart 6. She was able to induce nearly the optimum rate of growth in her young when the protein and inorganic contents of the corn kernel were supplemented by purified food additions. (See also chart 4, lot 1018.)

Chart 6. These records continue the series described in chart 5. Rat 844, whose diet consisted of corn and butter fat did no better in inducing growth in her young than did those in chart 5, where corn without any additions and with single additions other than fat-soluble A were fed. Both corn and wheat appear from the nursing records described as of distinctly better quality with respect to their content of fat-soluble A than is rolled oats. These records are in harmony with our studies on growth with these grains (10), (11), (12). The oat kernel contains proteins of distinctly better quality than are those of the wheat or corn kernels. This is shown by the fact that in the case of both wheat and corn both fat-soluble A and protein must be added before nearly normal milk could be produced, whereas in the case of the oat kernel, rat 1978 (chart 1), showed marked superiority in the quality of her milk as compared with rat 1943 (chart 4), whose ration consisted of wheat supplemented with salts and butter fat, and rat 1942 (chart 6), whose diet consisted of corn with the same supplements.

Rats 981 and 1942, when compared, demonstrate that for the production of normal milk the maize kernel must be improved with respect to both the protein and inorganic factors. The second periods in these two curves have little significance in the matter of showing anything about the quality of the milk which the mothers may have been producing at that time. It can, however, be readily seen from the growth of the young of rat 1017 (chart 6), that the quality of the milk produced is excellent when the diet of maize is supplemented with both protein and salts. In the second periods the young of rats 981 and 1942 should have been able to grow on the diets supplied them, without any aid in the way of supplemental milk feeding by the mother. These parts of the curves demonstrated clearly the capacity of the young to grow when the character of the diet was satisfactory.

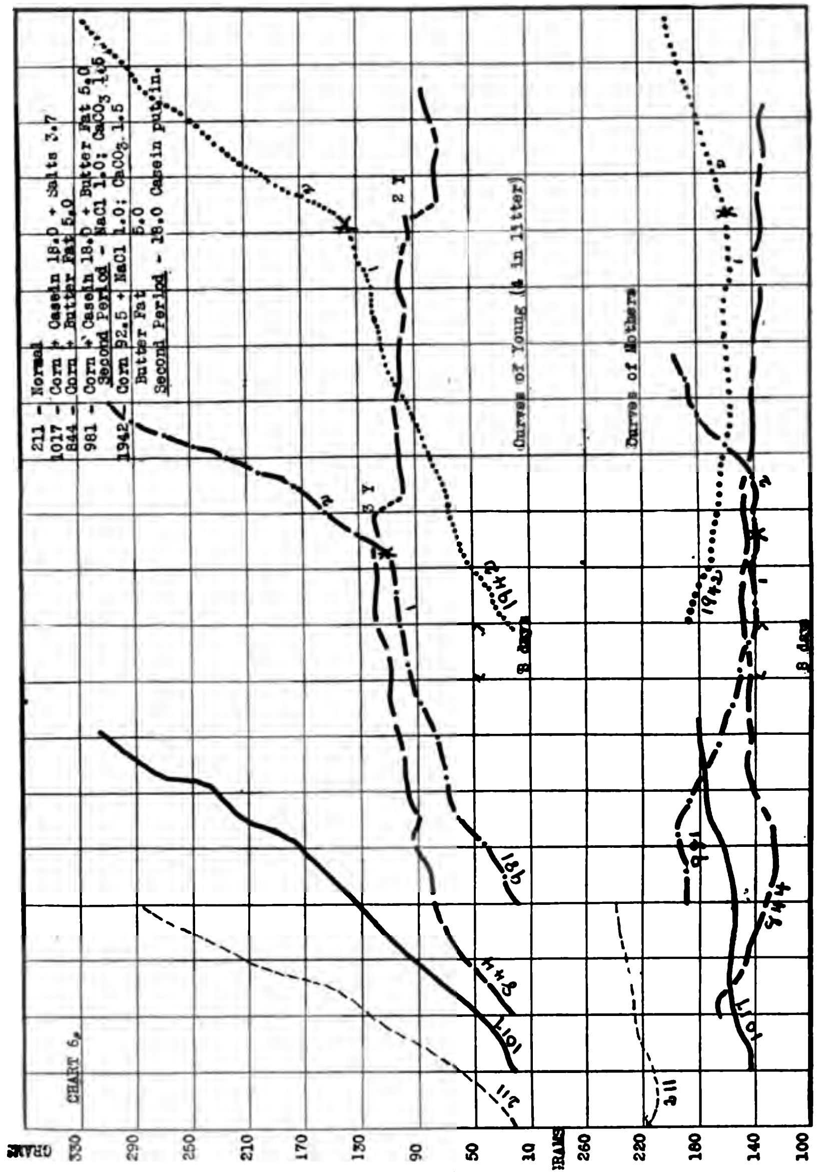
CHART 6.
211 - Normal
1017 - Corn + Casein 18.0 + Salts 3.7
844 - Corn + Butter Fat 5.0
981 - Corn + Casein 18.0 + Butter Fat 5.0
Second Period - NaCl 1.0; CaCO$_3$ 1.5
1942 Corn 92.5 + NaCl 1.0; CaCO$_3$ 1.5
Butter Fat 5.0
Second Period - 18.0 Casein put in.
Curves of Young (4 in litter)
Curves of Mothers
8 days
8 days
1 Y
2 Y
3 Y
211
1017
844
981
1942
GRAMS
330
290
250
210
170
130
90
50
10
GRAMS
260
220
180
140
100

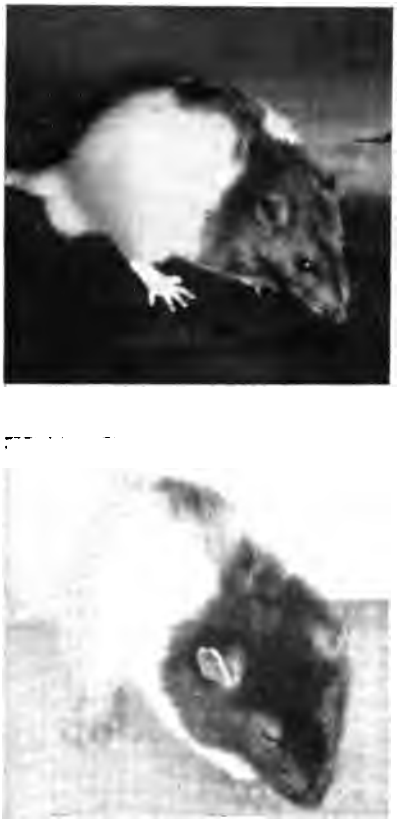

Fig. 1

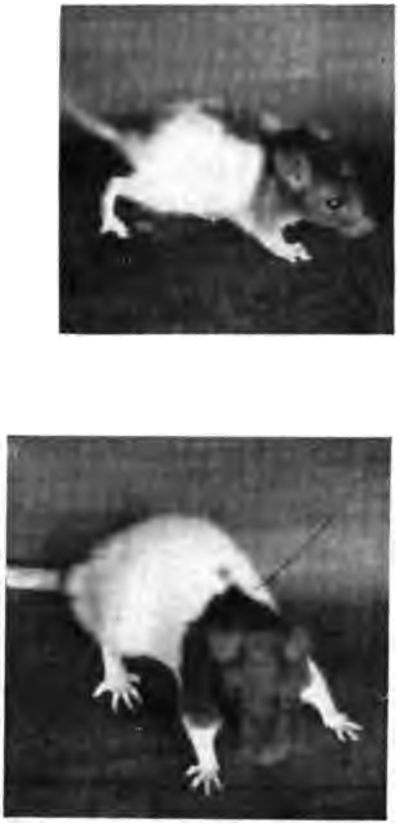

Fig. 2

Fig. 1. The picture above shows the appearance of the normal eyes of the rat. The picture below shows the condition of the eyes which is brought on by a lack of sufficient fat-soluble A in the diet. This we have described as a type of xerophthalmia.

Fig. 2. The rat above was 78 days old and weighed 52 grams when photographed. The diet of its mother was satisfactory in all respects, except that the quality of the protein was poor. The protein of a diet derived solely from seeds and seed products is inadequate for the formation of normal milk.

The rat below was 59 days old and weighed 160 grams when photographed. The milk of the mother was of excellent quality and supported rapid growth in the young.

Both animals had access to the food of their mothers as soon as they were able to run about. At the age of 25 days the rat above weighed 16 grams, that below weighed 45 grams.

SHOCK AND CIRCULATORY FAILURE FOLLOWING TRAUMA

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I. INTRODUCTION

In spite of the general impression that trauma and severe pain frequently lead directly to shock, there is still a great diversity of opinion as to whether experimental shock can be produced by these agents alone. Occasionally nerve stimulation or crushing the testes is reported to have been successful in producing shock in experimental animals (1). Most experimenters, however, finding that prolonged stimulation of nerves or infliction of trauma does not reduce the mean blood pressure to a low level, give up this mode of experimentation and resort to such measures as exposing of the intestines, clamping the inferior vena cava, etc.

Although exposure and handling of the intestines probably offer the most certain means of inducing circulatory failure, attention has been frequently directed to the fact that such drastic procedures are rarely if ever reduplicated in man, even in the most extensive laparotomies. Furthermore, the extreme congestion of the splanchnic vessels found in these conditions is apparently rare in those human cases of traumatic and surgical shock that come to operation (2). Small wonder, therefore, that the question is frequently raised whether the circulatory conditions thus inaugurated are comparable to those found in other forms of shock.

It was therefore decided to reinvestigate the question whether pain and trauma can produce shock in experimental animals and, if successful, to compare the dynamics of the circulation in this condition with that following intestinal exposure.

II. THE CRITERION OF CENTRAL NERVOUS SYSTEM SHOCK AND CIRCULATORY FAILURE

The term "shock" is now commonly employed, especially among laboratory investigators, to characterize a condition of low arterial pressure and an impaired peripheral circulation; in short, the term is used synonymously with "circulatory failure." There is strong evidence, clinical, experimental and in some cases morphological, that functional or physical damage to the central nervous system forms an important part of the picture to which the term "shock" is clinically given (3). The degree of involvement is apparently variable, depending on the nature, intensity and location of the injury. In trauma involving the spinal cord or brain, complete loss of consciousness and all the phenomena characteristic of spinal shock produced in the laboratory may be present. If the injury is peripheral the sensibility may be reduced, a state of apathy exist and the reflexes may be abolished, or these signs of central nervous involvement may be less extreme and all of the reflex reactions and even the muscular power may be normal. The latter is apparently the case in "wound shock" (4). In regarding the shock producing power of trauma applied peripherally, therefore, it is necessary to consider not only whether the blood pressure may be reduced to a low level in this way but whether demonstrable functional changes in the central nervous system take place. In order to avoid confusion the term "central nervous system shock" is employed as descriptive of a condition in which functional impairment of the central nervous system, recognizable by such signs as apathy, reduced or abolished sensibility and loss of reflexes, is present. To the dynamic circulatory changes which lead to an impaired blood-supply of the tissues and low blood pressures the term "circulatory failure" is applied. Upon this basis separate criteria as to whether the animal is in a condition of central nervous system shock and whether circulatory failure is present must be established.

A criterion for determining the presence of "central nervous system shock" is readily formulated. Since normal dogs, even after prolonged ether anesthesia (six to eight hours) recover from ether, as given by us, in less than fifteen minutes, we may safely consider "shock" involving the central nervous system present when, after discontinuing the anesthetic, the animals lie in a relaxed state several hours and when during this interval they fail to react to sensory stimulation.

The degree of circulatory involvement is more difficult to estimate. Complete circulatory failure is obviously present when the mean arterial pressure is exceedingly low and certainly imminent when the venous pressure has fallen markedly. Evidence is accumulating, however, that a very serious involvement of the circulation may be present without a marked reduction of mean arterial pressure. Circulatory efficiency, as correctly emphasized by Henderson (5), depends primarily on the volume of blood perfusing the organs. Of this the pressure curve recorded by the mean pressure manometer is an even less accurate indicator than is commonly appreciated. To determine the volume flow through all the organs of the body directly is not a simple procedure and can not be attempted without materially disturbing the circulation. In many cases, however, the pressure curves of the arteries, when optically recorded, are of differential value. A normal flow through the peripheral organs predicates that a considerable flow must continue during the relatively long interval of diastole. When, therefore, the decline of pressure (which indicates the rate of volume flow through the peripheral vessels for the major portion of the cardiac cycle) occurs only during systole, while the pressure declines very slightly or not at all during diastole, we may be certain that marked circulatory involvement exists, no matter what the mercury manometer registers.

III. METHOD AND PROCEDURE

In order to study the question whether pain and trauma *per se* are able to induce shock, it is necessary, on the one hand, to adequately control all accessory or contributory influences arising in the experimental method and, on the other, to make certain that conditions for producing shock are approximately as favorable as in man.

Anesthesia. It seems probable that the potency of peripheral stimuli and trauma in producing reactions in the body leading to shock and circulatory failure depends, to a considerable extent, on how little the conducting mechanisms of the spinal cord and brain are depressed by the anesthetic. Forbes and Miller (6), using the action currents as a guide, found that deep ether anesthesia abolishes or, at least, greatly reduces nerve impulses to the cerebrum. The anesthesia, therefore, should be light and only sufficient to abolish pain sensation. It is possible indeed that failure to induce shock readily by traumatic influences may in part be due to too deep an anesthesia. According to

Henderson and Haggard (7), light ether anesthesia must be cautiously employed, however, for the possibility exists that the attendant stimulation of respiration itself may result in shock. To control this factor the ether anesthesia in about half of the experiments was preceded by a small dose of morphine and deep breathing thereby avoided. Two experiments were performed under morphine anesthesia alone after the preliminary operation had been completed. Etherization in all experiments was carried out as follows: A large amount of ether was poured into a cone tightly wrapped with towels and the animal rapidly anesthetized. Tracheotomy was then quickly performed and, during the rest of the operation, ether was administered through the trachea by the closed method. Having completed the operative procedures required for attaching manometers to record venous pressures, intrathoracic pressure variations, mean arterial pressure and the optical tracing desired, the trachea was connected by inspiratory and expiratory valves, the former in partial circuit with a Brodie ether bottle. From now on fresh air admixed with ether was supplied for each inspiration and the expired air escaped by the expiratory valve. Observations were then immediately started.

As in the research recently communicated (8), the gross features of the circulation were followed by continuous records and observation of the respiration and mean arterial pressure. The differential venous pressure was at first followed by a differential water manometer but, as this was found to be absolutely erratic, sometimes even as regards directional changes of effective venous pressures, during and following the very excessive breathing brought about in these experiments, it was discarded. Instead, the effective venous pressure was determined as in former years (9) by algebraic addition of the right auricular pressure (read directly on a water manometer) and the intrathoracic pressure variations recorded by a calibrated tambour on the drum. For this reason the readings of effective venous pressures here reported are absolute and not merely relative as in a paper published recently (8). After a preliminary half-hour's observation of the normal circulation the testes were crushed periodically or the sciatic nerve was dissected out and the central end stimulated for approximately two minute intervals between which a pause of thirty seconds was allowed.

IV. EFFECTS ON THE REACTIONS OF THE CENTRAL NERVOUS SYSTEM

Crushing of the testes and spermatic cord and stimulation of the sciatic nerves caused (with one exception in twenty-one experiments) a very intense effect both on the depth and rate of respirations. In no instance, even after intensely deep breathing had continued for the greater part of two hours, did permanent apnea or death from respiratory failure, such as reported by Henderson (10) after intense artificial respiration, take place. Following the deep breathing a very short period of apnea occurred or the respirations became temporarily shallow. Continued periodically for a time interval ranging from forty minutes to one and one-half hours, this left most of the animals in a state of "central nervous system shock." This occurred in sixteen out of the twenty-one experiments; it was impossible to bring the remainder to this condition. The percentage is sufficiently large to conclude that the symptoms of "central nervous system shock" may be induced in this way. More in detail, the animals lay quietly when the anesthetic had been removed and failed to react to auditory, touch, temperature or pain stimuli. In several animals, after more than an hour had elapsed a sciatic nerve was dissected out and clamped, with no reaction or objection on the part of the animal. During this stage the winking reflex was present, the pupils were dilated, lachrymation and salivary secretions were profuse.

Of the sixteen experiments in which this state of shock supervened the outcome was fatal in seven, death being due to accidental causes in two. The other five may be said to have died of "shock." All of these fatal cases followed crushing of the testes, while none of the animals in which the sciatic nerve alone was stimulated succumbed. The remaining nine animals eventually recovered. In these shock was produced by crushing the testes in two animals; by sciatic stimulation in two and by combined crushing and stimulation in five. The mode of recovery was interesting. In most experiments evidence of recovery became manifest within two to four hours. It began with an increase in rate and depth of respirations and, in some instances, some slowing of the heart. The animal then reacted more and more readily to painful stimuli applied to the forelegs and thorax but remained unresponsive to similar stimuli applied to the posterior portions of the body. Gradually these also were effective and finally the animal began to show signs of consciousness. This stage usually required about an hour and at that time it was obviously necessary to discontinue observations on the state of the circulation. Gradually voluntary movements reappeared, first in the fore- and then in the hind-legs. The dog then sat up and walked about, responded to calling and in some cases ate or drank. Several dogs in which the trachea and neck operations were surgically repaired survived until the next day and ate at that time. They were then killed.

V. CIRCULATORY CHANGES IN SHOCK

Circulatory changes, of course, were inaugurated by each infliction of trauma and upon stimulation of the sciatic nerves. The nature of these reactions is well known to physiologists. Sciatic stimulation promptly produces an elevation of mean arterial and effective venous pressures. The latter is undoubtedly due largely to the mechanical effect of deep breathing aided, perhaps, by the reflex cardiac slowing. The rise of mean pressure is predominantly due to a reflex vasoconstriction. Crushing of the testes likewise increases the effective venous pressure through the mechanical influence of augmented breathing. The mean arterial pressure, however, lowers, owing largely to the reflex vasodilation.

Considering the central nervous system effects produced by sciatic stimulation and crushing experiments in the light of these early circulation disturbances, it is obvious that the most serious involvement of the central nervous system is produced only when the peripheral vessels are primarily dilated and a passive anemia of the central nervous system is present.

The same holds true as regards the degree of circulatory involvement of the five animals that failed to develop "central nervous system shock." Stimulation of the sciatic nerve was used exclusively in three cases and a combination of sciatic stimulation and crushing of testes was utilized in the other two. In these cases the circulatory changes upon cessation of the noxious influences were not affected to any marked extent.

The sixteen animals that developed reactions of the central nervous system characteristic of shock may be grouped as follows, according to their circulatory condition:

A. Five animals died in shock. Four of these showed circulatory changes closely resembling those described in the case of progressive and complete circulatory failure following intestinal exposure. One animal showed no marked alteration of the venous or arterial pressure but toward the end the circulation rapidly failed from an acute cardiac insufficiency.

B. Two animals showing pronounced degree of "central nervous system shock" and a considerable reduction of venous and arterial pressure died from accidental causes. These must be left out of consideration.

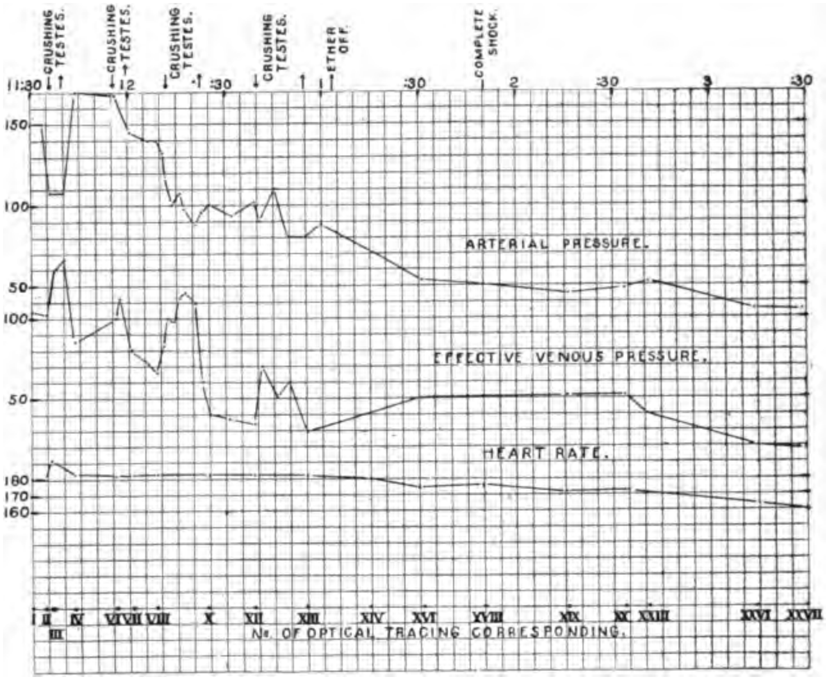

Fig. 1. Chart showing progressive changes in arterial pressure, effective venous pressure and heart rate following repeated crushing of testes and leading finally to death. Roman numerals refer to optical records of arterial pressure taken at corresponding points. Some of these are shown in figure 2.

C. Nine animals showed marked signs of "central nervous system shock" from which they subsequently recovered. All of these animals showed a distinct involvement of the circulation, although complete circulatory failure was at no time present. A more detailed analysis of the hemodynamics follows:

Shock accompanied by complete circulatory failure and terminating fatally. The gross changes of the circulation of one experiment are detailed in the plot

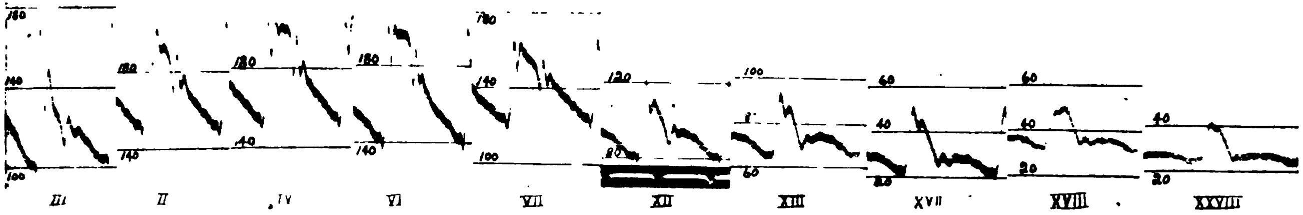

Fig. 2. Segments of pressure variations in central arteries taken at different stages of fatal circulatory failure by a calibrated optical manometer. Roman numerals correspond to points indicated on chart of figure 1.

of figure 1 and a few segments of the optical, arterial tracings corresponding in number to the points marked on the plot are shown in figure 2. For thirty minutes after the operation had been completed the mean arterial pressure averaged 150 mm. and the normal contour of the arterial pressure curve did not alter. Crushing of the testes was begun at 11.36 a.m. and was repeated at short intervals, as indicated by arrows on the plot of figure 1.

Every application of violence resulted in (1) a fall of mean arterial pressure; (2) a marked acceleration and augmentation of respiration with great expiratory effort; (3) an increase in the absolute and effective venous pressures. The latter occurred whether venous pressure was high or low, thereby demonstrating the mechanical ability of deep breathing to augment venous pressure. At first the fall of arterial pressure was accompanied by cardiac acceleration. The optical records obtained at this time show evidence of decreased arterial filling (fig. 2). The amplitude is greater, the primary wave larger and the systolic portion declines more rapidly (fig. 2, *II* and *III*). Inasmuch as the effective venous pressure actually increased during the stimulation and the heart accelerated, the diminished filling of the arterial trunks can be attributed only to a reduction in peripheral resistance probably reflex in character. After cessation of a single (or sometimes several) attempt at crushing the testes, the respiration became slower and shallow, mean arterial pressure returned to a level above normal and the optical curves regained their normal contour (fig. 2, *IV* and *VI*).

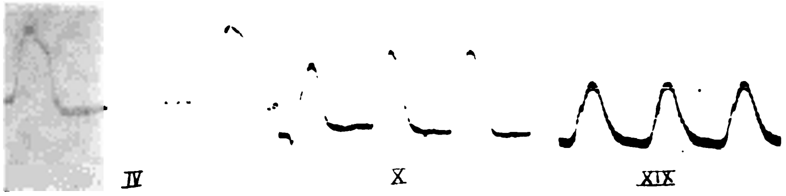

Fig. 3. Three segments of optically recorded pressure variations from right ventricle taken from the case of shock summarized in table 1. Roman numerals correspond to those of this table.

Following frequent repetitions, however, the mean pressure recovered less and less and the effective venous pressure became progressively lower. The optical tracings (fig. 2, *VII* to *XIII*) show a progressive decrease in amplitude, indicating that the cardiac discharge was impaired. The heart became progressively slower. At 12.54 p.m. crushing was discontinued and the animal was left unmolested. Both the mean arterial and effective venous pressures continued on their downward course. Ether was discontinued at 1.03 p.m. and the animal never again reacted to painful stimuli until death occurred at 4.17 p.m. The optical curves are shown in segments *XVII*, *XVIII* and *XXVIII* of figure 2. Post mortem examination showed the intestinal loops to be pale and the veins poorly filled.

This experiment is typical of few cases in which trauma alone or in combination with sciatic stimulation rapidly produced a condition of shock accompanied by circulatory failure recognizable by the fall of mean arterial and venous pressure alone. That the circulatory failure in these cases is similar to the cases of abdominal shock previously reported and predominantly of cardiac origin, is further suggested by the changing contour of the pressure curves optically recorded for the right ventricle. This is shown in an experiment from which the curves reported in figure 3 are taken. The tabular summary of this experiment is appended.

TABLE 1

Experiment C-165. January 25, 1918. Dog under ether anesthesia

TIME	BLOOD PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.00		108	180	I	Ether started
12.15	128	110	170	III	
12.55	106	Higher			Crushing testes
12.56	120	120	202	IV	
1.03	112	120	165	V	
1.05					Ether off
1.07	116		160	VI	
1.09	136	130	170	VIII	
1.22					Ether on
1.57	120	80	216	X	After stimulating sciatic 15 minutes
2.07	116	133	216	XI	Sciatic stimulation
2.12	92	128			Sciatic stimulation stopped
2.27	106	60	260	XIV	Complete shock present
2.31	100	50	222	XV	
2.45	50	55	216	XVI	
3.03	40	35	202	XVII	
3.17	46	35	202	XVIII	
4.18	44	40	214	XIX	
4.50					Death

The circulatory dynamics in shock followed by recovery. Complete circulatory failure recognizable by a marked fall of effective venous or mean arterial pressure never occurred in any of the nine dogs that temporarily developed a complete state of "central nervous system shock" but recovered therefrom. In some instances, in fact, the arterial pressure was above 120 mm. during the state of shock. This indicates clearly that a low arterial pressure is not an essential condition of "central nervous system shock." The further conclusion that shock may occur without circulatory involvement is not justified, however, for when carefully studied it is found that in this condition significant and often dangerous alterations of the circulation exist. These facts are illustrated in the following experiment:

TABLE 2

Experiment C-168. January 24, 1918. Anesthesia, light ether

TIME	BLOOD PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.18	142	40	120		
11.25	140	40	120		
11.33	150	70	117	III	
11.38*	132	90	165	IV	After crushing testes
11.40	150	82	112	V	
11.44*	108	160			Crushing testes
11.48	151	66			
11.54*	126	160			Crushing testes
11.56*	160	130			Crushing testes continued
12.00*	140	120			Crushing testes stopped
12.05	128	70	160	VII	
12.13	132	72			
12.18*	150	120	230	IX	Crushing testes
12.35*	106	160			Crushing testes
12.39	118	120	230	XII	After crushing testes
12.50*	98	130	240	XIV	Crushing testes
1.00*	90	140			End of crushing
1.03	101	130	240	XVI	Animal in central nervous shock
1.40	126	102	165	XVII	
3.00	123	45	165	XIX	
3.35					Dog shows slight reaction from sensory stimuli; signs of recovery from shock
4.55					Animal conscious—reacts to sound

* Observations made during infliction of trauma.

During each crushing act the mean arterial pressure fell markedly and, owing largely to the mechanical result of the vigorous respiratory effects, the effective venous pressure was markedly elevated. If the mean pressures following the periods of relative rest between crushings alone are considered, the curve has a slightly downward course but never falls below 100. The corresponding curve of effective venous pressure gradually mounted, however, so that when the direct mechanical effect of the augmented breathing ceased (e.g., at 1.40 p.m.) the venous pressure still remained considerably elevated. Reduction of venous

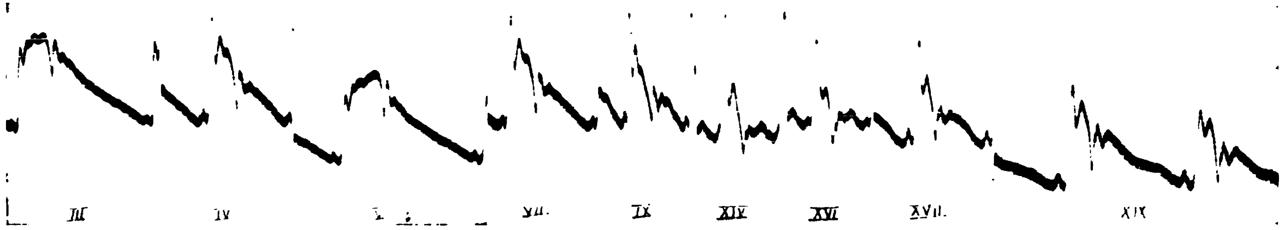

Fig. 4. Segments of optically recorded arterial pressure changes from a case of "central nervous system shock" followed by recovery. Mean arterial pressure was sustained and effective venous pressure was elevated, as shown in tabular summary of table 2. Roman numerals correspond to those in this table.

pressure, therefore, scarcely played a rôle. It was true in six out of the nine cases which recovered that the effective venous pressure was moderately increased during the stage of shock. The heart progressively accelerated. A consideration of the optical tracings shows, however, that the arterial circulation was materially altered. This is shown in the segments of figure 4. A detailed analysis is scarcely necessary. The series of curves shows that, as in the initial stage characteristic of abdominal shock, the filling of the arterial trunks becomes progressively reduced. This reaches its climax at the end of the crushing period to which curve *XVI* corresponds. With each systole the blood column is thrown vigorously and the pressure drops more and more rapidly during the latter portion of systole. Following the incisura and throughout diastole the pressure is very low and declines but little, indicating that the peripheral flow is largely limited to systole. As the effective venous pressure is higher than before and the cardiac rate is increased, this can be accounted for only by a reduced peripheral resistance. From this condition recovery slowly sets in until forty minutes later when curves such as are shown in segment *XVII* are recorded and by the time the animal begins to react slightly to sensory stimulation the normal characteristics again gradually begin to develop. In this case a bigeminal heart beat followed, as shown in segment *XIX*. This was clearly recognized by auscultation and disappeared an hour later. These cases show that whenever "central nervous system shock" develops as a result of trauma it is always accompanied by distinct circulatory changes in the nature of a prolonged low peripheral resistance. The mean arterial pressure is apparently maintained by the cardiac acceleration but, as is evident from the pressure curves of figure 4, is no longer a reliable index of the circulation.

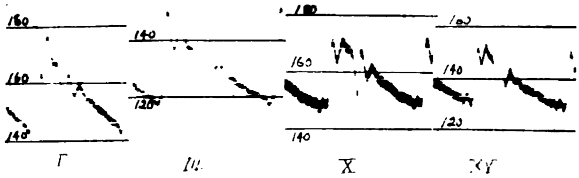

Fig. 5. Segments of optically recorded arterial pressure changes following prolonged sciatic stimulation and resulting in temporary central nervous system involvement. Details shown in table 3.

Circulatory changes following prolonged sensory stimulation and resulting in temporary central nervous system shock. In two cases in which shock with subsequent recovery occurred as a result of prolonged sciatic stimulation, the mean arterial pressure was maintained at a level above the original but the effective venous pressure was gradually reduced to a moderate degree. The data from an illustrative experiment are shown in the following table:

TABLE 3

Experiment C-171. February 4, 1918. Anesthesia, ether without morphine

TIME	ARTERIAL PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.53	145	74	174	I	
11.56–12					Crushing testes
12.02	116	78	146	III	After crushing testes
12.14	182	170			Stimulating sciatic
12.44	144	50	193	IV	After stimulation stopped
12.50	134	42	186	VI	
12.58	138	44	180	VII	
1.37	157	46	160	VIII	
1.42–1.55					
2.03	166	42	174	X	
2.27	152	40	174	XI	
2.31–2.39					Sciatic stimulation
2.40					Animal in shock
2.48	150	30	174	XIII	
3.18	140	38			
3.28	160	34	174	XIV	
3.34	154	34			
4.43	130	46	174		
4.50	146	46	174	XV	
5.20					Animal shows reaction to sensory stimulation
5.48					Conscious; struggles

The nature of the optical tracings corresponding to certain phases of the condition are shown in figure 5. It is evident that they are affected largely as regards amplitude, the pulse pressure decreasing and showing less essential changes in contour. The pressure at the beginning of diastole remains relatively high and the slope of the diastolic limb remains gradual. Contrary to the other cases, the lowered venous pressure and reduced cardiac discharge were compensated by an increased peripheral resistance. It seems that the pressor effect of stimulating the sciatic nerve becomes permanent in these cases, while the depressor effect of crushing the testes predominates in the others.

VI. CONCLUSIONS

1. A state of shock involving the central nervous system can be produced experimentally by trauma. This state may persist from two to five hours, after which recovery sets in; or it may be fatal.

2. Prolonged sensory stimulation may cause a temporary depression of the functions of the central nervous system but in itself does not lead to permanent changes or death.

3. "Central nervous system shock" never occurs without circulatory involvement which is always clearly indicated in optically recorded pressure curves from the arteries but is not necessarily evident in the mean pressure variations as given by the mercury manometer.

4. In the milder cases of shock; i.e., in those terminating in recovery, the circulatory derangement corresponds essentially to that described as characteristic in the initial and early progressive stage of circulatory failure in abdominal shock. Optical arterial pressure tracings show that a diminished volume of blood is contained in the arterial trunks and that the peripheral flow is thereby reduced. In most instances this is solely due to a reduction of the total arterial resistance while the effective venous pressure becomes somewhat increased through the mechanical effects of prolonged deep breathing. In a few cases only was the effective venous pressure reduced somewhat and constituted the main cause of arterial depletion.

5. In severe forms of shock; i.e., in those terminating fatally, the initial stage in which reduced peripheral resistance plays a rôle is of short duration, the effective venous pressure falls early, reaches a low level and by reducing the cardiac discharge, is the chief cause of complete circulatory failure.

6. The dynamic changes of the circulation which lead to progressive and complete circulatory failure are not essentially different in shock produced by trauma and that produced by intestinal exposure. The differences, if any, are in degree and duration of the respective phases but not in the character of the disturbance.

7. Considering all the available evidence, two factors may be said to be concerned in circulatory failure accompanying shock: *a*, the reduction of peripheral resistance; and *b*, the fall of effective venous pressure, decreasing the systolic discharge.

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EFFECTS OF EXTERNAL TEMPERATURE, MORPHINE, QUININE AND STRYCHNINE ON THYROID ACTIVITY

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All investigators are ready to grant that the thyroid gland is capable of quantitative variations of function and some maintain that under abnormal conditions it may, as well, vary qualitatively. The more these controlling factors become evident, the clearer will be our understanding of the true physiology of the thyroid gland.

Seidell and Fenger (1) demonstrated a seasonal variation in the iodin content of the thyroid glands of cattle, sheep and hogs, the amount of iodin being greatest in the late summer while the minimum was reached in February and March. Since it has been found by Marine and Lenhart (2) and others that the stainable colloid of the thyroid varies directly with the iodin content of the gland and inversely with the degree of hyperplasia, it is probable that this seasonal variation in the iodin content of the gland represents a variation in the activity of the gland itself. Thus in the summer the gland would tend to assume a resting type with a storage of colloid material while in winter a degree of hyperplasia would develop with a corresponding diminution in the iodin content. In producing this seasonal variation in the activity of the gland different factors might play a part: Variations in the composition of the food at the different seasons; difference in the climatic conditions of the various seasons other than temperature differences; and differences in the temperature alone.

Cramer (3) observed that while considerable histological variations are found in the thyroids of rats living under natural but experimentally uncontrolled conditions, these variations disappear when the animals are housed in a warm room kept at a constant temperature of 20° to 25°C., carefully handled and fed regularly on a suitable diet. He found the gland vesicles then to be somewhat distended with a well-staining colloid and lined with cubical epithelium. By exposing the animals to cold for several days he found that the gland vesicles became collapsed, the lining epithelium became columnar and the colloid lost its affinity for certain stains. This would seem to indicate that changes in the thyroid gland similar to those occurring in its seasonal variations can be induced by artificially regulating the temperature of the environment. It was with the idea of discovering just how extensively variations in this one factor of the environment would serve to produce changes similar to those resulting from seasonal variations that the present work was undertaken.

Also it was desired to know whether, in external temperature variations, we could not have here a means of producing at will either an active or resting type of thyroid. Many attempts have been made to find agencies which would produce such changes and some degree of success has resulted, more so with producing hyperplastic changes than with reducing the activity of the gland. Hunt (4), C. Watson (5), Marine (6), Bensley (7) and Burget (8) all found that hyperplastic changes could be induced by altering the diet of the animals, especially by increasing the protein percentage of the diet. Mansfield and Ernst (9) and Martin, Loevenhart and Bunting (10) obtained such changes as a result of a decreased oxygen supply to the animals. Regarding the resting type of thyroid, Marine (11) obtained distinct changes in that direction almost at will by means of iodin medication, while many workers have produced like results by thyroid feeding. Jackson (12), by subjecting animals to periods of inanition, produced changes in the epithelial cells of the thyroid similar to those usually taken as indicating decreased activity of the gland. Therefore it was thought that it would be of value if it could be found that changes in either direction could be produced at will simply by variations in the temperature of the environment.

METHODS

Young growing rabbits were mainly used in order that there might be some measure of their basal metabolism as evidenced by their rate of growth. However, adult rabbits were also used as well as guinea pigs and cats, in order to show that the findings were not peculiar to young rabbits.

The animals were kept in a room with a fairly constant temperature of 12° to 18°C., and fed regularly on a constant diet for several days or sometimes even weeks before beginning the treatment in order to bring the glands as nearly as possible to a uniform condition in each case. At the end of the pre-experimental period a control specimen of the thyroid of each animal was removed aseptically under ether anaesthesia, fixed, sectioned, stained and kept for comparison with the specimen taken from the same gland after the treatment. For the effects of cold these same animals were placed in a cold room or out-of-doors at a temperature of −5° to + 10°C. and kept there for periods of time varying from three to thirty days. For heat treatment a well-ventilated hot-box with a temperature of 27° to 37°C. was used, with the duration of the treatment varying as above. At the end of the experimental period a portion was removed from the thyroid of each animal, prepared as above and compared with the first specimen taken from the same animal.

Differences in the histological appearance of the secreting cells and the colloid of the gland were considered in this work to represent differences in the activity of the gland at the time the specimens were taken.

Von Orth's fixative was used on the tissues at first but it was found that alcohol-formol fixative (10 per cent formalin in 80 per cent alcohol) gave practically the same results so far as this work was concerned, so the latter fixative was used for most of the tissue specimens.

A record of the daily weights and rectal temperatures of the animals was kept in several series. The diet was kept constant during pre-experimental and the experimental periods.

TABLE 1

ANIMAL	DAYS OF TREATMENT	TEMPERATURE	COLLOID	APPEARANCE OF CELLS	
		°C.		Before	After
Rabbit 10......	10	30–36	Increased	Cuboidal	Flattened
Rabbit 39......	16	31–39	Increased	Cuboidal	Flattened
Rabbit 42......	16	31–39	Increased	Cuboidal	Flattened
Rabbit 49......	8	31–34	Increased	High cuboidal	Flattened
Rabbit 50......	8	31–34	Increased	High cuboidal	Slightly flattened
Cat 9...........	3	30–35	Increased	Low columnar	Medium to low cuboidal
Cat 14..........	15	28–35	Increased	Low columnar	Cuboidal
Guinea pig 2....	10	27–38	Increased	Cuboidal	Low cuboidal

RESULTS

a. Effects of high external temperatures. The above table of results indicates the changes produced in eight typical examples taken from the total list of thirty three animals subjected to high temperatures. Twenty eight rabbits, two cats and three guinea pigs were treated in this way and in this total there were only four instances in which no changes in either the character of the cells or colloid content of the vesicles were noted. In no case was there either a decrease in the amount of colloid or an increased height of the epithelial cells. Typically, the changes were as follows:

TABLE 2

ANIMAL	DATE	EXTERNAL TEMPERATURE	RECTAL TEMPERATURE	WEIGHT
		°C.	°C.	grams
Rabbit 49	October 25	18	38.9	980
	October 26	31–34	39.1	940
	October 27	31–34	39.8	990
	October 28	31–34	40.3	915
	October 29	31–34	39.1	965
	October 30	31–34	40.3	975
	October 31	31–34	39.1	955
	November 1	31–34	41.4	960
Rabbit 21	June 2	18	38.9	720
	June 3	12–15	No marked variations	800
	June 4	12–15		810
	June 5	12–15		815
	June 6	12–15		850
	June 7	12–15		850
	June 8	12–15		930
	June 9	12–15		930
	June 10	12–15		925
	June 11	12–15		945
	June 12	12–15		985
	June 13	12–15		1000
Rabbit 9	May 6	18	39.0	575
	May 8	15–18	No marked variations	570
	May 11	15–18		615
	May 13	15–18		650
	May 14	15–18		650

The colloid of the vesicles increased in amount, became more uniform in appearance and took on the stain more readily. Vacuoles were often present in the outer portion of the colloid mass, more especially in the glands of the animals used during the winter months. In almost every instance these vacuoles disappeared entirely or decreased in size and number during the treatment. The epithelial cells lining the vesicles usually became more flattened, with nuclei and cytoplasm more densely staining, and in no instance were the cells observed to be increased in height.

Accompanying these microscopic changes in the thyroids, there were also other changes in the animals. If the temperature of the hot-box was kept at 30° to 35°C., it was found that growth in these animals was much slower than in the controls, that the fur became dull and unkempt and that the appetite seemed to decrease as the temperature increased. When the temperature of the box was raised so as to be equal to or slightly above the body temperature of the animals and kept at that level for a few days, a loss in weight and emaciation occurred probably as a direct result of the loss of appetite. Death often followed if this condition was continued for very many days. In such instances as these there also occurred a rise in the rectal temperatures of the animals of 0.5° to 2.0°C., which might also have been a factor in producing the loss in weight and emaciation.

TABLE 3

ANIMAL	DAYS OF TREATMENT	TEMPERATURE	COLLOID	APPEARANCE OF CELLS	
				Before	After
Rabbit 3.....	17	−10 to + 7°C.	Decreased	Low cuboidal	Medium cuboidal
Rabbit 44....	21	−5 to +10°C.	Decreased	Flattened	High cuboidal
Rabbit 51....	30	−5 to +10°C.	Decreased	Flattened	Cuboidal
Rabbit 53....	10	−5 to +10°C.	Decreased	Cuboidal	Columnar
Rabbit 54....	6	−5 to +10°C.	Decreased	Cuboidal	Low columnar
Guinea pig 6.	27	−10 to +10°C.	Decreased	Low cuboidal	High cuboidal
Guinea pig 7.	27	−10 to +10°C.	Decreased	Low cuboidal	High cuboidal

The effects of high external temperatures on the growth rate and on the body temperatures of the animals are shown in table 2.

b. Effects of low external temperatures. Table 3 summarizes the histological changes in the thyroids of seven of the twenty animals subjected to low temperatures. Sixteen rabbits and four guinea pigs were treated in this way and in every instance there resulted either a decrease in colloid with an increased vacuole formation or a heightening of the epithelial cells, or both together. The results given in the table are typical of those obtained in the whole series of animals subjected to low temperatures.

The changes here are just the reverse of those described as resulting from high external temperatures. The colloid decreased in amount, lost its smooth uniform character and showed an increase in the amount of vacuolation. The epithelial cells of the vesicles were increased in height while the nuclei and cytoplasm were not nearly so densely staining as before. The rate of growth of the animals was much greater than that of the animals kept in the hot-box and seemed also to be increased as the temperature of the environment was lowered below normal, as is indicated in table 2. However, more work is being done at present to determine the extent of these effects on growth produced by both high and low temperatures. The fur of the animals became thick and fluffy and their excitability appeared to be very high, apparently indicating a high degree of vitality. The rectal temperature was maintained at normal.

TABLE 4

Quinine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 25...	4	7	60–120	Increased	Low columnar	Cuboidal
Rabbit 26...	2	3	60–150	Increased	Low cuboidal	Same
Rabbit 31...	4	8	90–240	Increased	Cuboidal	Low cuboidal—flattened
Rabbit 32...	4	8	90–240	Increased	Low cuboidal	Same
Rabbit 35...	4	8	60–150	Increased	Medium cuboidal	Low cuboidal
Rabbit 36...	4	8	60–150	Increased	High cuboidal	Medium cuboidal

c. Effects of quinine, morphine and strychnine. Since changes in the external temperature seemed to affect metabolism as judged by body weights and conditions and thyroid activity as judged by morphology, the attempt was next made to influence metabolism directly by the action of drugs to determine whether the activity of the thyroid was correspondingly influenced. The pre-experimental period was observed as before while the external temperature and diet were kept practically constant throughout the experimentation.

Quinine was used for its action on the endogenous metabolism and was administered as quinine bisulphate dissolved in normal salt solution at 37°C., 60 mgm. per cubic centimeter, injected intramuscularly. The results obtained are indicated in the following table.

Thus it is seen that quinine treatment resulted in changes that are practically identical with those observed under high temperatures, so far as the histological changes indicate. Follicular colloid increased in amount, the epithelial cells decreased in height and both cells and colloid became more densely staining.

TABLE 5

Morphine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 27.....	5	10	30–90	Increased	Cuboidal	Low cuboidal
Rabbit 28.....	3	4	30–60	Increased	Cuboidal	Low cuboidal
Rabbit 29.....	4	7	60–150	Increased	High cuboidal	Low cuboidal
Rabbit 30.....	4	7	60–150	No change	High cuboidal	Low cuboidal
Rabbit 33.....	4	8	30–90	No change	High cuboidal	Medium cuboidal
Rabbit 34.....	4	8	30–90	No change	Cuboidal	Same
Rabbit 44.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 45.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 46.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 47.....	11	16	30–90	Increased	Cuboidal	Flattened
Guinea pig 6..	3	5	90–120	Increased	High cuboidal	Low cuboidal
Guinea pig 7..	3	5	90–120	No change	High cuboidal	Same

Morphine was used as morphine sulphate dissolved in normal salt solution at 37°C., 60 mgm. per cubic centimeter, injected hypodermically. The dosage was so adjusted as to cause the animals to lie quietly with shallow respiration for two to three hours following each injection. The results of the treatment are given in table 5.

Here again the changes are seen to be the same as those observed in the thyroid resulting from high temperatures, namely, a tendency to become a resting type of gland.

Strychnine was tried in a few cases and some changes were observed as illustrated in table 6 below. However, due to the rapid excretion of the strychnine and the rather narrow margin between the stages of hyperexcitability and convulsions, the results were not very satisfactory. The drug was injected subcutaneously in a solution of 0.2 mgm. per cubic centimeter for rabbits and 1 mgm. per cubic centimeter for guinea pigs. The attempt was to so adjust the dosage as to just produce a condition of hyperexcitability and repeat it as often as possible without causing convulsions. The injection should have been repeated every one to one and a half hours but other work kept this schedule from being followed as closely as it should have been. For that reason the results are probably less marked than they would have been under more ideal conditions.

TABLE 6

Strychnine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 57.....	5	22	0.3–0.4	No change	Cuboidal	Same
Rabbit 58.....	5	22	0.3–0.4	No change	Flattened	Medium cuboidal
Rabbit 59.....	5	22	0.3–0.4	Decreased, more vacuolated	Cuboidal	Low columnar
Rabbit 60.....	5	22	0.3–0.4	Much more vacuolated	Flattened	Cuboidal
Guinea pig 11.	5	20	1–1.5	More vacuolated	Low cuboidal	Medium cuboidal
Guinea pig 12.	5	20	1–1.5	More vacuolated	Low cuboidal	Medium cuboidal
Guinea pig 13.	10	40	1–1.5	No change	Low cuboidal	Medium cuboidal
Guinea pig 14.	1	1	1.5	More vacuolated	Flattened	Cuboidal

It will be seen from the table that the vesicles did not change in size during the treatment except in one instance where there was a distinct decrease in the amount of colloid. However, in all but two of the animals the vacuoles in the outer part of the colloid masses of the thyroid increased in size and number quite noticeably. In all but one instance there occurred an increase in the height of the epithelial cells lining the vesicles. It would seem that here we have a more active type of gland as a result of the strychnine treatment.

DISCUSSION AND CONCLUSIONS

The morphological changes in the thyroid described as resulting from the different forms of treatment in these experiments, are accepted by most observers as indicating variations in the activity of the gland. Although Bensley holds that the colloid in itself is no indication of the activity of the gland, still changes in the amount and character of the colloid and in the appearance of the secreting cells surely serve to demonstrate changes in the activity of the gland during the experimental period. Thus, under high temperatures we see a storage of colloid material in the vesicles together with such changes in the secreting cells as are usually thought to represent a diminished secretory activity. Therefore it is to be concluded from the results of these experiments that an elevation in the external temperature serves as an agency for reducing the activity of the thyroid glands of animals.

However, the mode of physiological adaptation by which these changes in the gland are brought about is not definitely understood. Accompanying the decreased thyroid activity there is a slowing of the endogenous metabolism of growth and also probably of the metabolism for heat production, since heat elimination is made much more difficult by the high external temperature. The interrelation of these three phases is still a subject for speculation.

Means and Aub (13) and DuBois (14) found that thyroid feeding can markedly increase basal metabolism, as measured by the calorimeter, while thyroid deficiency, as in cretin patients, produces a basal metabolic rate much below normal. Mansfield and Ernst (9) have shown that fever in normal animals (dogs and rabbits) is accompanied by increased protein metabolism and also increased total metabolism, while in thyroidless animals there is no increase in metabolism of either kind, the fever being due only to a decreased heat loss. It seems then that the thyroid is closely concerned with all metabolic processes, those for heat production as well as those for protein metabolism.

In the case of high external temperatures, then, the first effect is probably a diminished heat production or exogenous metabolism, caused perhaps by reflex inhibition of the thermo-genetic mechanism, including the thyroid. The lessened growth rate observed in the animals might then be due to the decreased thyroid activity. There are probably other factors which aid in producing these results, perhaps the activity of the parathyroid and suprarenal glands. However, this work did not include observations on these structures.

In animals subjected to low temperatures there are found histological changes in the thyroid which indicate an increased activity of the gland. Also there occurs a rapid rate of growth together with a seeming hyper-irritability and general condition of high vitality. Since the rectal temperatures of the animals remained normal in the cold environment, there must have been a great increase in the heat production. Thus the process is probably just the reverse of that resulting from high temperatures. The thermo-genetic mechanism is stimulated by the low temperature of the surroundings and, since the thyroid seems to be included in this mechanism, its activity would thus be increased. This increased thyroid activity then very likely causes more rapid growth metabolism, thus accounting for the rate of growth.

By the action of quinine on the animals it was possible to influence principally the endogenous metabolism and observe the results of this on the thyroid activity. As the results indicate, there occurs a diminished activity of the gland. Since quinine has no known action directly on the thyroid, it seems that the depressed protein metabolism must cause the decreased thyroid activity, probably by bringing about a diminished need for the secretion in the body and allowing its storage as colloid in the gland vesicles.

Morphine produces results in the thyroid similar to those following quinine treatment, although probably by a different action of the drug. This drug also has no known action directly on the thyroid but seems to act on metabolism mainly by reducing nervous sensibility and consequently all metabolic processes dependent on nervous influence, especially muscular activity. The thyroid gland, being closely related to total metabolism, is probably then inhibited by the lessened need for its secretion.

Strychnine, although having no known action on the thyroid directly, seems to cause an increased activity of the gland in these experiments. This is probably due to the greatly increased metabolism in the muscles resulting from the action of the drug on the spinal cord, a larger amount of thyroid secretion being needed for this increased oxidation.

The fact that, the vacuoles in the outer part of the colloid masses of the thyroid varied so uniformly in these experiments seems to me to be of some importance. Low temperatures and strychnine treatment, resulting in an increased thyroid activity, also caused an increase in the number and size of these vacuoles in almost every case. Likewise high temperatures, morphine and quinine caused a diminution in the vacuolation. Whether the vacuoles represent areas from which the colloid has been resorbed to supplement the new secretion of the gland in supplying the body needs or whether it represents the deposit of some new substance by the epithelial cells, are questions yet to be answered. The behavior of the vacuoles under the different conditions in these experiments seems to favor the first theory.

SUMMARY

High external temperatures cause a diminished activity of the thyroid glands of animals, as judged by morphology, together with a slowing of the rate of growth.

Low external temperatures, on the contrary, increase the thyroid activity and also seem to cause a faster rate of growth.

Morphine and quinine appear to decrease the activity of the gland, probably as a result of the lessened metabolism and diminished heat production. Strychnine, on the other hand, causes greater thyroid activity, very likely by increasing metabolism through its action on the spinal cord.

NOTE.—This work was begun in the physiological laboratory of the University of South Dakota, under the direction of Dr. A. L. Tatum. I wish here to express my appreciation of his interest and many helpful suggestions during the course of the work under his direction. I also wish to thank Dr. O. O. Stoland for his interest in the work as it was carried on at the University of Kansas.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLVII. Gastric Secretion and Urine Ammonia

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Hawk (1) in his series of studies on water drinking has found an increase in urine ammonia on increasing the amount of water during the meals, which he ascribes to a stimulation of gastric secretion. It had been shown by Walter (2) and others that the urine ammonia can be increased or decreased by the feeding of acids or alkalies. Schittenhelm (3), working upon patients with gastric "hypo-and hyperacidity." came to the conclusion that "an increase in the acidity of the gastric juice causes increased ammonia excretion." A. Loeb (4) and Gammeltoft (5), working with patients and normal persons, report some instances of increased urine ammonia after the meal but a greater number in which there was a decrease in ammonia excretion after the meal. The latter investigator also reports a marked decrease in the urine ammonia upon taking sodium bicarbonate with the meal.

The work presented in this paper is an attempt to throw more definite light upon the relation between gastric secretion and urine ammonia.

GENERAL METHODS

Effort has been made to control all factors such as diet, time since the last meal and the water intake, that might influence the excretion of ammonia. Gastric analyses were made every fifteen minutes. Urine was collected in fifteen to thirty minute intervals by catheter in dogs and by voluntary micturition in man. With training and practice on the part of both man and dog, the accuracy of these methods of urine collection cannot be questioned. Controls were made for one-half to one hour preceding the experiment to determine the continuous gastric secretion and ammonia excretion. The work has been done on five men and has been repeated on female dogs with gastric and duodenal fistulas and exposed urethra. Both the Folin and the Folin-

Nessler (6) methods have been used in the determination of ammonia. The determination was always made on fresh urine. Conclusions are based on from three to ten trials of the same experiment in each individual.

TABLE 1

PROCEDURE 400 cc. H_2O WITH MEAL	GASTRIC JUICE		URINE		PROCEDURE 800 cc. H_2O WITH MEAL	GASTRIC JUICE		URINE	
	Free	Total	Amount	NH_3 N		Free	Total	Amount	NH_3 N
a.m.			*cc.*	*mgm.*	*a.m.*			*mgm.*	*cc.*
6.00	15*	20.0*			6.00	12.5	20.0		
6.30	17.5	25.0	12	4.0	6.30	15.0	25.0	15	2.1
Meal 7.00	17.5	25.0	11	4.2	Meal 7.00	18.0	27.5	12	2.3
7.30	0	5.0	28	5.0	7.30	0	5.0	28	4.2
8.00	0	17.5	48	7.2	8.00	0	12.5	72	8.2
8.30	1.0	25.0	90	8.4	8.30	5.0	22.5	140	11.0
9.00	1.0	32.5	44	8.0	9.00	10.0	40.0	40	8.7
9.30	17.5	47.5	30	6.0	9.30	22.5	80.0	32	4.5
10.00	25.0	82.5	28	4.6	10.00	40.0	100.0	24	5.2
10.30	40.0	100.0	20	4.2	10.30	67.5	115.0	16	4.8
11.00	45.0	105.0	20	4.0	Stomach 11.00	75.0	110.0	10	4.1
					empty at				
11.30	60.0	95.0	13	3.0	11.15 11.30	60.0	75.0	11	4.1
Stomach 12.00	45.0	55.0	12	2.5	12.00	37.5	45.0	9	4.3
12.30	30.0	40.0	13	2.7	12.30	27.5	35.0	8	4.0

* The free and total gastric acidity is expressed in clinical units, which is the number of cubic centimeters of N /10 Na OH required to neutralize 100 cc. of gastric contents.

EXPERIMENTAL

Gastric stimulation with absorption in the intestine and urine ammonia

Digestion and absorption of a standard meal with "moderate" and "copious" amounts of water with the meal. The subject, with normal gastric history, was on a diet with a standard meal consisting of 125 grams of graham crackers, 50 grams of peanut-butter, 300 cc. of milk and 400 cc. of water. When 400 cc. of water were ingested with the meal the amount was called "moderate," while the amount was called "copious" when 800 cc. were drunk. Five cubic centimeters of the stomach contents were taken out for each determination of acidity.

The results as recorded in table 1 are typical, in the case of this individual, for a diet[1] that lasted nine days. The increase in ammonia after the meal along with the diuresis is marked. When the amount of water with the meal was increased to 800 cc., the ammonia excretion was increased. During the period of increased water drinking, the total daily amount of ammonia was increased by 40 mgm., which corroborates the findings of Wills and Hawk (1) and is shown in table 2.

Not only does table 2 show an increase in urine ammonia upon increasing the water with the meal but it also shows that a greater increase takes place when the water ingested with the meal is increased, although the total daily amount of water intake is the same. Referring to table 1, it is seen that the increased excretion of ammonia occurs during the period of the absorption of the water, indicated by the increase in urine amount. Although there is a greater gastric stimulation when 800 cc. of water are drunk than when 400 cc. are drunk, likewise when 200 cc. are drunk, it might be suggested, since the urine ammonia was not very decidedly increased, that the increase is due to the absorption of the excessive water or to the diuresis. (Observations to answer this suggestion have been made and will be presented later in the paper).

TABLE 2

Daily ammonia excretion as influenced by water drinking with the meals

CUBIC CENTIMETERS OF H_2O WITH MEAL	PERIOD II (3 DAYS) MODERATE H_2O (1800 CC.) MILLIGRAMS OF NH_3 N	PERIOD III (3 DAYS COPIOUS H_2O (3600 CC.) MILLIGRAMS OF NH_3 N	PERIOD IV (3 DAYS) COPIOUS H_2O (3600 CC.) MILLIGRAMS OF NH_3N
200	240*		
800		294*	
400			281*

* Average daily amount for the three day-periods

Digestion and absorption of a standard meal. The three subjects in this experiment were not on a standard diet but did ingest the standard meal mentioned above. The results in tables 3 and 4 are typical for a series of six tests performed upon each individual. Table 5 shows typical results for three tests performed on two dogs on a diet of one

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[1] The diet was conducted as follows: A preliminary period of two days without control of amount of water; a period of three days of moderate water (1800 cc.) taking 200 cc. with the meal; a third period of three days of copious water (3600 cc.) taking 800 cc. with the meal; a fourth period of three days of copious water (3600 cc.) taking 400 cc. with the meal. See table 2.

TABLE 3

PROCEDURE	SUBJECT N				SUBJECT I			
	Test I		Test IV		Test II		Test VI	
	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour
p. m.		*mgm.*		*mgm.*		*mgm.*		*mgm.*
1–4	97	15.8	25	32.0	30	12.1	36	14.3
Meal 5.00	32	16.8	26	29.4	49	18.2	47	19.1
6.00	50	18.2	33	30.8	98	23.8	75	21.6
7.00	73	23.4	37	28.6	35	23.8	53	22.4
8.00	35	28.0	36	29.4	28	16.0	32	18.7
9.00	29	23.4	31	29.4	20	14.1	18	14.3
10.00	24	22.5	28	28.5	15	12.4	14	11.5

TABLE 4

PROCEDURE	SUBJECT K				
	Urine amount	NH_3 N per hour	Time	Urine amount	NH_3 N per hour
a. m.		*mgm.*	*p.m.*		*mgm.*
11.00–12.00	54	8.4	4.00–5.00	50	19.6
Meal 12.30	23	4.3	Meal 6.00	37	14.1
1.00	19	4.3	7.00	50	28.0
1.30	70	9.6	8.00	77	32.5
2.00	260	11.2	9.00	38	28.0
2.30	52	6.3	10.00	26	21.0
3.00	34	9.8	11.00	18	18.2
4.00	49	12.6			
5.00	42	8.9			

TABLE 5

PROCEDURE	DOG A		PROCEDURE	DOG B	
	Urine amount	NH_3 N per hour		Urine amount	NH_3 N per hour
		mgm.			*mgm.*
11.30–2.45	51	42	9.10– 9.40	3	4.4
3.15	5	5.4	10.10	3	4.9
Meal 3.45	4	6.1	Meal 10.40	4	5.8
4.15	7	9.5	11.10	5	10.5
4.45	13	19.6	11.40	14	16.6
5.15	22	26.6	12.10	19	17.15
5.45	18	16.8	1.10	42	36.6
6.15	11	15.4	2.10	40	37.0
			3.10	30	30.0
			4.10	15	18.0

meal a day of 200 grams of fresh lean meat ground and mixed with 250 cc. of H_2O.

Subject N, as table 3 shows, gave varying results. This individual, although normal and in excellent health, never gave with an Ewald meal (fractional method) a higher acidity than 0.16 per cent; and with water there was practically no stimulation of the gastric glands. This is offered as an explanation why increase in ammonia output did not occur with this person. Subject H, table 4, always showed an increase in urine ammonia after a meal as did subject I. Both of these individuals gave a marked response to water stimulation and the Ewald meal. Marked diuresis generally occurred. Why the dogs show such a very marked increase in urine ammonia (table 5) after the meal, has not been determined. But it has been observed as a general observation, without any direct evidence other than that shown in this paper, that the "NH_3 mechanism" in the dogs is more easily influenced by acids and alkalies than in the case of the men worked upon.

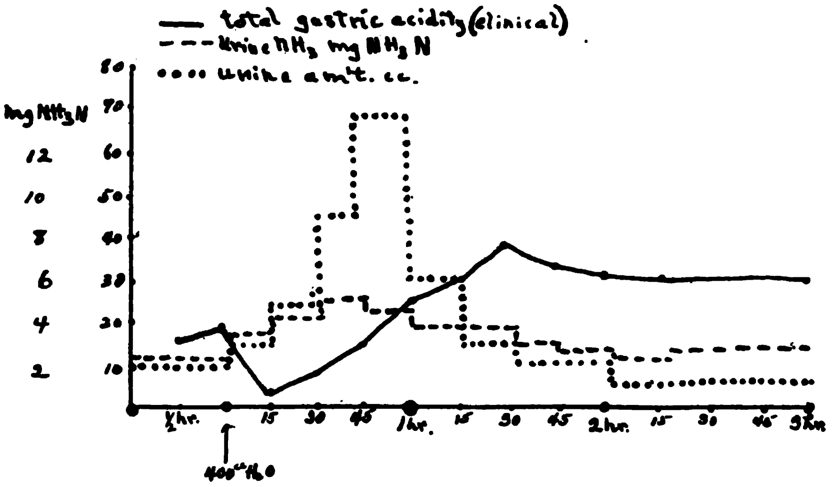

Fig. 1

Gastric stimulation with water: man and dog. In a series of experiments upon five men, the urine was collected and the continuous gastric secretion was withdrawn previous to the ingestion of the water, after which urine was collected every fifteen or thirty minutes. Four hundred cubic centimeters of tap water were given by mouth and samples were withdrawn every fifteen minutes for analysis. The above figure (fig. 1) shows the composite of ten tests upon one individual.

Although these curves (fig. 1) show an increase in urine ammonia, a diuresis and a slight gastric stimulation, it was recognized that allowing the water to remain in the stomach obscured the true relation between these factors. So it was decided to increase the amount of water used to 700 cc. and to empty the stomach completely at the end of every fifteen minutes, thereby desiring to obtain an increase in gastric response, a closer relation of the curves, a diuresis and at the same time have a measured quantity of water pass into the intestine. The following curve (fig. 2) shows the composite of ten tests upon the same individual used in figure 1.

In the ten tests an average of 500 cc. were withdrawn from the stomach after the end of fifteen minutes. This water was generally slightly bile tinged, (Gmelin's test) the latter portion withdrawn being more so. Although this curve shows no great increase in urine ammonia, it is significant because of its constancy and its occurrence when, as shown by controls, the urine ammonia would otherwise be on the decline. This person always showed a decline in urine ammonia during the early morning and forenoon until the mid-day meal was eaten, provided that no water or food was taken in the meantime.

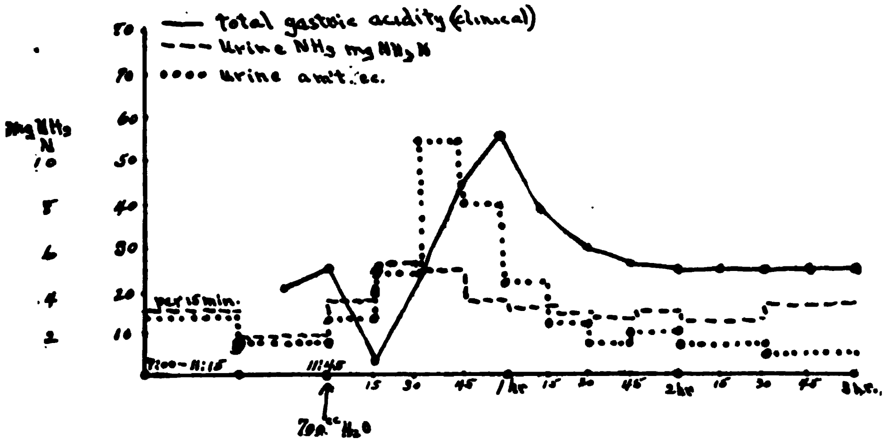

Fig. 2

This experiment was repeated upon four other normal men. In two of these the stomach[2] was stimulated by water and the urine ammonia was increased. In the other two there was practically no response to water and no increase in NH_3 excretion resulted. Figure 3 shows the

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[2] Work upon this question has shown that all stomachs of apparently normal persons are not stimulated by water. There seems to be some relation between the emptying time and the occurrence of the stimulation, e.g., those stomachs that empty less than 150 cc. in fifteen minutes when 400 cc. are drunk, respond much more than those stomachs that empty more than 150 cc.

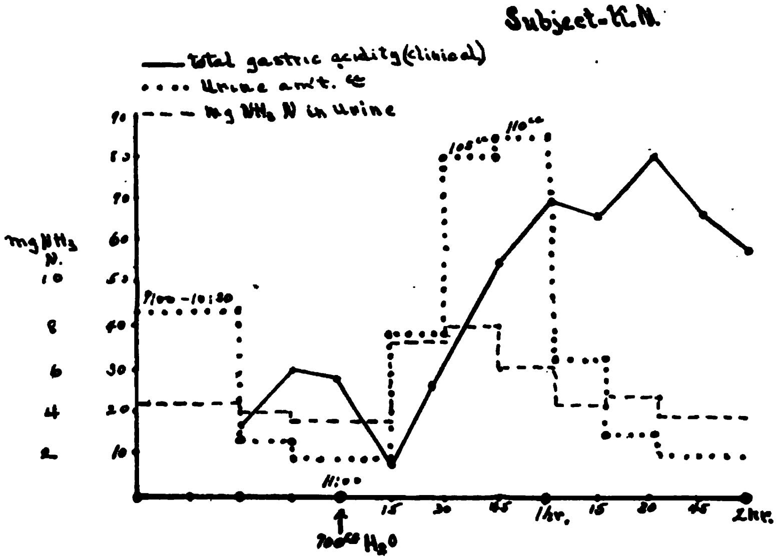

Fig. 3

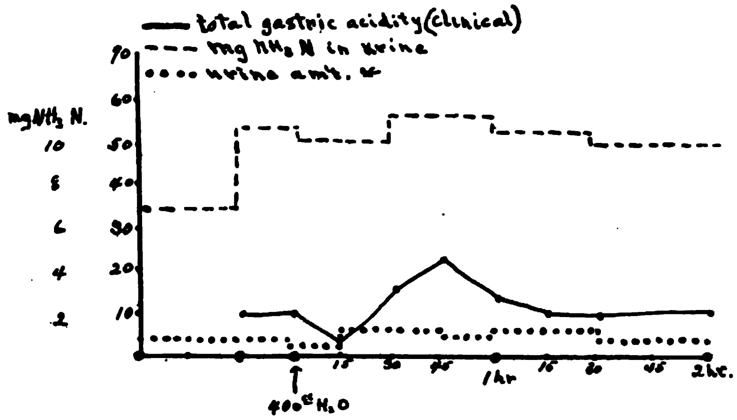

Fig. 4

response in a man whose stomach was stimulated by water, and figure 4 shows the response in a man whose stomach was practically not stimulated by water.

This work was repeated upon four dogs with the urethra exposed for catheterization and similar results were obtained as upon man. Three of the dogs showed increased urine ammonia upon water ingestion, while the fourth showed no change. In the three dogs that did show increased NH_3 excretion it was known that their stomachs responded to stimulation by water as these dogs possessed Pavlov accessory stomachs and had been used in other work. The fourth animal died of distemper before it was found whether the stomach responded to water stimulation. The dogs were trained to take water by stomach tube without being disturbed thereby.

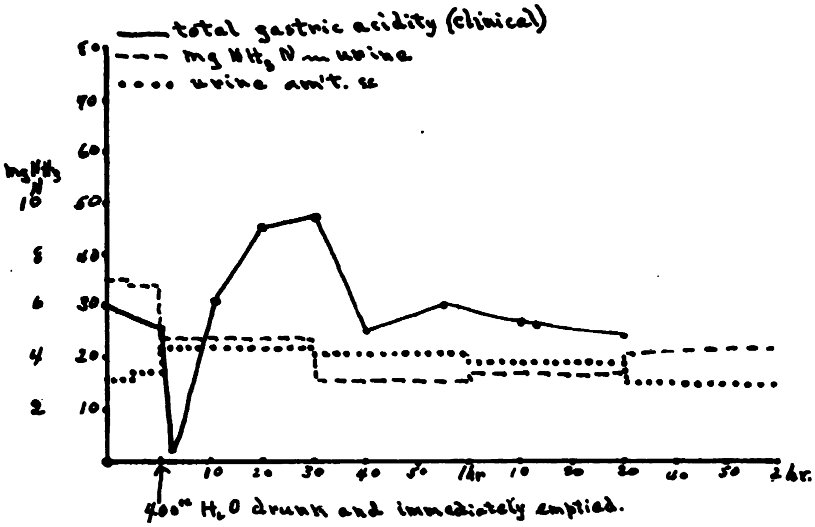

Fig. 5

It is apparent, then, that most of the cases in this series show an increase in urine ammonia upon gastric stimulation followed by absorption in the intestine and that where this increase in urine ammonia does not occur, it has been shown that the acidity of the gastric juice was low or gastric stimulation did not occur.

Gastric stimulation without absorption in the intestine and urine ammonia

Stimulation by water: man. In these experiments 400 cc. of water were drunk and immediately pumped out. The gastric juice resulting from the stimulation was drawn out so as to prevent it from passing into the intestine. The amount of fluid withdrawn was measured and compared with the original amount to make sure that some had passed into the duodenum. This process of drinking the 400 cc. of water and emptying it from the stomach never required longer than one and a half minutes. Figure 5 is a typical example of the results obtained.

Gastric stimulation by meat broth: man. The same procedure used above in gastric stimulation by water was used here also. Four hundred cubic centimeters of meat broth were drunk and immediately withdrawn from the stomach. The results are shown in figure 6.

Figures 5 and 6 show a gradual fall in urine ammonia upon stimulation of the stomach. This occurred constantly in the one person worked upon. This result is not as significant as it might appear, however, for this individual always showed a decline in urine ammonia during the period of the day in which these experiments were conducted. It does show that there is no increase in urine ammonia upon gastric stimulation.

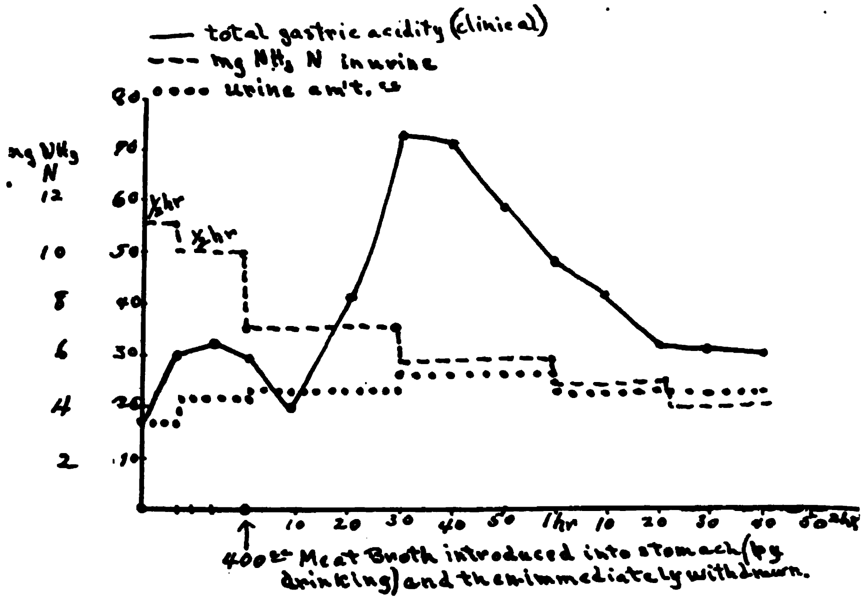

Fig. 6

Gastric stimulation by food: dog. Gastric digestion without absorption. Two dogs[3] with gastrostomy and duodenostomy and perineorrhaphy

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[3] If the fistula in the duodenum is made too large, the dogs will not live more than 8 to 11 days. They die of general weakness and debility. The duodenum shows generally pin point ulcers and some enteritis, neither being very extensive

(to expose urethral orifice in order to facilitate catheterization) were fed 100 grams of lean finely ground cooked meat with 100 cc. of water. The food as it left the stomach was conducted from the duodenum, about one and a half inches from the pyloric sphincter, by means of a glass cannula. At times it was found convenient to use an aspirator which was connected to the glass cannula. The emptying time for this meal was generally two and one-half hours (this varied in different dogs) and the amount of chyme collected was generally 75 cc. to 100 cc. more than the amount of semi-fluid ingested. These dogs were dieted during the experimental period.

TABLE 6

Showing urine NH_3 *during gastric digestion without absorption*

PROCEDURE	DOG: PUP, ON DIET				DOG: WHITE, ON DIET			
	Meal: 100 grams meat—100 cc. H_2O		Control, no meal		Meal: 100 grams meat—100 cc. H_2O		Control, no meal	
	Urine	NH_3 N	Urine	NH_3 N	Urine	NH_3 N	Urine	NH_3 N
a. m.	*cc.*	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*
7.00–7.30	9	4	11	6.2	12	6.0	15	8.3
Meal 8.00	8	3.8	10	6.0	10	5.6	13	8.0
8.30	6	3.0	8	5.2	9	5.2	14	8.0
9.00	6	3.1	8.5	6.0	6.5	4.7	12	7.6
9.30	5	2.6	7	5.0	6	4.7	11	7.8
10.00	5	2.6	7	4.8	6	4.2	9	7.0
10.30	4	2.0	7	4.8	5	3.9	9	7.0
11.00	4	2.5	6.5	4.5	4	3.1	8	6.4
11.30	4	2.5	7	4.5	4	3.1	8.5	6.7

or sufficient per se to cause death. Nausea is easily produced by manipulation of the duodenal mucosa. In some instances in dogs that I have observed, when fluid was injected into the distal portion of the gut, a large amount was pushed back through the fistula, unless it was stopped by plugging the fistula with cotton. Violent vomiting was often produced by this latter method and was only relieved by taking the cotton from the fistula and permitting the fluid to flow out. If the fistula is made small (size of a pencil or about 1 cm. in diameter) the dogs will live indefinitely in good health and will not vomit upon injecting fluid, if the latter is injected slowly.

At Dr. A. B. Luckhardt's suggestion this operation has been done in two stages. In the first operation the gastrostomy was made and the first 3 to 4 inches of the duodenum transplanted extraperitoneally. In the second operation a small opening (1 cm. in diameter) was made in the duodenum. Dogs thus operated will live indefinitely. The opening must be dilated daily to prevent closure.[1]

Here again a decrease in the urine ammonia is noticed but a control also shows a gradual decrease. The only conclusion warranted, then, is that there is no increase in urine ammonia during gastric digestion.

Gastric stimulation without absorption followed by the absorption of acid and neutral chyme

Acid chyme: dog. The dogs with the gastric and duodenal fistulas were used in this experiment. The foregoing experiment with them was repeated and the chyme, instead of being thrown away was collected, and when the stomach was empty, it was injected into the distal portion of the intestine for absorption. The chyme titrated from 0.18 per cent to 0.22 per cent total acid and from 0.05 per cent to 0.1 per cent free acid. The free acidity was obtained only toward the latter part of digestion. Tables 7 and 8 show typical results of three trials on each dog.

TABLE 7

Dog P: on diet. Acid chyme. Trial III

TIME	URINE AMOUNT	NH_3 N	REMARKS
a.m.		*mgm.*	
7.00–7.30	2.5	3.2	
8.00	2.2	2.2	Meal: 100 grams ground, cooked meat, 150 cc. H_2O
8.30	2.2	2.2	
9.00	2.2	2.2	
9.30	2.0	2.2	
10.00	1.8	1.7	
10.30	1.8	1.8	Stomach empty; injected the chyme collected into duodenum
11.00	6.5	8.3	
11.30	8.0	12.8	
12.00	10.0	11.0	
12.30	7.0	8.8	
1.00	5.5	7.0	
1.30	4.0	5.1	
2.00	3.0	4.1	

Table 7 shows the slight decline in urine ammonia during digestion referred to before, followed by a marked increase in the urine ammonia and the quantity of the urine output upon the injection of the acid chyme.

Injection of neutral chyme. The foregoing experiment was repeated but instead of injecting the acid chyme, the chyme was made neutral to phenolphthalein by the addition of $NaHCO_3$.

Table 8 shows that the absorption of neutral chyme did not increase the urine ammonia, although a diuresis resulted. On the other hand, there is a diminution of NH_3 after the injection, which is of some significance as it occurred in each of the dogs and in all of the three trials on the same dog.

It is apparent from the results of the injection of acid and neutral chyme into the intestine for absorption that there is an increase in the urine ammonia upon the absorption of the acid gastric contents and no increase in NH_3 upon the absorption of neutral gastric contents.

TABLE 8

Dog P: on diet. Neutral chyme. Trial II

TIME	URINE, AMOUNT	NH_3 N	REMARKS
a. m.		*mgm.*	
7.00–7.30	4.0	4.2	
8.00	4.5	4.0	Meal: 100 grams ground, cooked meat, 150 cc. H_2O
8.30	4.0	4.4	
9.00	3.5	3.8	
9.30	3.5	3.8	
10.00	3.2	3.6	Stomach empty at 10.15. Chyme was neutralized with $NaHCO_3$ and injected into duodenum
10.30	3.0	3.2	
11.00	8.0	3.2	
11.30	12.5	2.0	
12.00	9.2	1.4	
12.30	6.0	1.3	
1.00	4.0	1.3	

Influence of the consistency of the acid chyme upon urine ammonia

As it is well known that water is absorbed in the intestine at a rapid rate and as digestion of food substances by enzymes takes place more rapidly and completely in dilution, thereby facilitating absorption, it was considered of importance to ascertain if the fluid consistency of the chyme had any influence upon urine ammonia.

In one of the duodenal fistula dogs the semi-fluid chyme was collected and the supernatant liquid decanted. This liquid was titrated and its total acidity calculated. The amount of N/2HCl equal to this total acidity was then added to the solid portion of the chyme so that the amount of the acid in the solid chyme was now equal to the amount of the solid chyme plus the supernatant liquid. The solid chyme was then injected into the duodenum. Table 9 shows typical results of the three trials of this experiment.

As was expected and as is shown by the results in table 9, the consistency or dilution of the acid chyme is a factor in the increase in the urine ammonia during absorption. On diluting the acid chyme the urine ammonia was increased as compared with the absorption of more solid chyme, although the total acid content was kept the same. In the latter no free acid was present at all. By way of explanation, dilution facilitates digestion and absorption; and free acid is present. As a result acid is thrown into the blood at a more rapid rate and more ammonia is required to neutralize the acid in order that the H-ion equilibrium be maintained without drawing upon the plasma alkaline reserve.

TABLE 9

Consistency of acid chyme and urine ammonia. Dog P: on diet. Trial III

TIME DECEMBER 24 AND 25	CONTROL: A 325 CC. SEMI-FLUID CHYME		100 CC. OF SEMI-SOLID CHYME B		REMARKS
	Urine, Amount	NH_3 N	Urine, Amount	NH_3 N	
a. m.		*mgm.*		*mgm.*	
8.00					Meal: 100 grams ground cooked meat, 15 cc. H_2O
8.30	8.0	5.3	8.5	3.7	
9.00	7.0	4.7	7.0	3.5	
9.30	6.2	4.5	6.5	3.0	
10.00	6.0	4.0	6.3	3.0	Stomach empty: A, 10.00; B, 10.25. Injected into duodenum
Inj. 10.30	5.8	3.5	5.0	2.5	
11.00	6.0	6.7	5.5	4.8	A, 325 cc. chyme fluid, total acidity 140 cc. N/10HCl*
11.30	13.0	11.2	6.0	6.9	
12.00	9.2	8.5	5.0	8.0	B, 100 cc. semi-solid chyme, total acidity 152 cc. N/10HCl*
12.30	7.6	6.3	4.5	5.1	
1.00	6.1	6.0	4.0	4.5	
1.30	6.0	5.8	4.0	4.5	

* All of this acidity is not of gastric juice origin as the meat fed was acid.

Absorption of water, acid and alkali from the intestine

In these experiments the subject would swallow at night before retiring two tubes, one of flexible rubber of such a length that it would not be passed into the duodenum, and the other a tube of the Einhorn

TABLE 10

Absorption of water from the intestine: man. Trial II

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.30–7.00	25.0	25.0	32.5	20	6.0	
7.15	10.0	32.5	40.0			
7.30	9.0	27.5	37.5	15	5.1	
7.45	7.5	27.5	37.5			
Inj. 8.00	8.0	25.0	35.0	14	5.0	Injected 200 cc. H_2O into duodenum via duodenal tube
8.15	7.0	22.5	30.0			
8.30	9.0	22.5	32.5	20	4.8	
8.45	6.0	20.0	30.0			
9.00	7.0	20.0	32.5	25	4.6	
9.30	10.0	22.5	30.0	20	4.0	
10.00	12.0	17.5	25.0	18	4.0	
10.30	9.0	20.0	27.5	16	3.5	

TABLE 10A

Absorption of water from the intestine: dog. 150 cc. H_2O

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
2.00			2.00			
2.30	8	5.6	2.30	5.0	2.8	
Inj. 3.00	7	5.0	Inj. 3.00	4.5	3.2	Injection made via the duodenal fistula
3.30	8	6.0	3.30	6.0	3.3	
4.00	12	6.0	4.00	10.0	3.6	
4.30	16	6.4	4.30	16.0	3.6	
5.00	20	5.6	5.00	14.0	4.0	
5.30	14	4.8	5.30	12.0	3.6	
6.00	10	4.5	6.00	7.0	2.5	

type of such a length that 15 inches could pass into the duodenum. The next morning the longer tube would be in the duodenum, which could be accurately determined by blowing air into the tube, or still more accurately by applying suction to the tube in the stomach while injecting fluid into the duodenum. If the fluid injected was drawn out through the stomach tube, the duodenal tube was not in the duodenum but in the stomach. Failure of the tube to pass into the duodenum occurred only once during a series of thirty tests. Urine was collected in half hour intervals and the continuous gastric secretion withdrawn every fifteen minutes. Each experiment with acid, alkali and water was repeated three times.

TABLE 11

Absorption of acid from the intestine: man. Trial III

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH$_3$ N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.30–7.00				13.0	10.8	
7.15	50.0	20.0	35.0			
7.30	10.0	30.0	35.0	12.5	11.0	
7.45	7.0	32.5	40.0			
Inj. 8.00	8.0	32.5	40.0	12.5	9.8	Injected 200 cc. N/20 HCl into duodenum[*]
8.15	7.5	30.0	40.0			
8.30	7.0	35.0	40.0	19.0	10.8	
8.45	8.0	0	10.0			Bile regurgitation[†]
9.00	8.0	2.5	12.5	18.5	13.0	
9.30	10.0	35.0	47.5	17.0	11.0	
10.00	11.0	40.0	47.5	16.0	9.8	
10.30	10.0	32.5	37.5	15.0	8.2	
11.00	9.0	30.0	37.5	10.0	7.6	

[*] There was a regurgitation of bile into the stomach in every trial. A clamp had to be placed on the duodenal tube to prevent bile being forced out upon the clothing.

[†] This increase in ammonia is not very marked but is significant as it occurred during a time when the urine ammonia was normally on the decline, as stated before for this individual. The largest increase observed during the series of four experiments was 4 mgm. per fifteen-minute period. This table is not published as the gastric record is incomplete.

Tables 10, 11, 12 and 13 will show typical results obtained upon one man with the injection of water, acid and alkali.

TABLE 11A

Absorption of acid from the intestine: dog. 150 cc. N/20 HCl

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
1.30	contents		1.30	contents		
2.00	6	4.2	2.00	4.0	5.1	
2.30	5	4.0	2.30	4.0	4.8	
Inj. 3.00	5	4.0	Inj. 3.00	4.0	5.1	Injection made via duodenal fistula
3.30	7	4.8	3.30	8.0	7.6	
4.00	14	9.6	4.00	12.0	8.0	
4.30	20	15.1	4.30	15.0	10.1	
5.00	25	13.2	5.00	19.0	8.5	
5.30	18	8.6	5.30	17.0	9.4	
6.00	12	7.2	6.00	12.5	7.6	
6.30	9	6.1	6.30	7.0	6.2	

TABLE 12

Absorption of alkali from the intestine: man. Trial I

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.00–6.30	36	20.0	37.5	15	8.9	
7.00	14	35.0	45.0	15	8.8	
7.15	7	35.0	42.5			
Inj. 7.30	7	32.5	40.0	20	10.0	Injected 200 cc. 5 per cent $NaHCO_3$ into duodenum
7.45	20	0	1.0			Bile
8.00				19	2.0	Vomited 30 cc. of bile*
8.15						
8.30	20	0	10.0	26	1.8	
8.45	5	0	12.0			
9.00				32	1.6	Stomach dry: no continuous secretion†
9.15						
9.30	5	0	17.5	38	3.0	
10.00	5	7.5	20.0	28	3.0	
10.30	5	15.0	25.0	28	3.0	

* Nausea is very marked and in two instances caused deep abdominal vomiting, expelling tubes and bile. Regurgitation of bile into the stomach always occurred.

† This inhibition resulted only one time.

TABLE 12A

Absorption of alkali from the intestine: dog. 150 cc. 5 per cent $NaHCO_3$

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
2.00	contents		2.00	contents		
2.30	4	5.4	2.30	6	4.6	
Inj. 3.00	3	5.0	3.00	5	4.6	Injection made via duodenal fistula
3.30	6	4.2	3.30	7	4.0	
4.00	9	2.0	4.00	10	1.8	
4.30	12	0.8	4.30	14	1.2	
5.00	18	1.0	5.00	8	1.0	
5.30	10	0.8	5.30	7	0.8	
6.00	7	1.0	6.00	5	0.8	

TABLE 13

Absorption of water from the large intestine: man. Trial IV

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a.m.	*cc.*			*cc.*	*mgm.*	
9.45–10.15	4.0	12.5	17.5	16.2	5.0	
10.30	4.0	12.5	17.5			
Inj. 10.45	4.0	12.5	20.0	18.5	4.8	Injected per enema of 750 cc. warm H_2O into colon*
11.00	20.0	20.0	22.0			
11.15	8.5	20.0	22.0	16.2	5.3	Bile tinged
11.30	8.0	15.0	20.0			
11.45				13.0	3.0	
12.00	18.0	30.0	32.5			
12.15	5.5	25.0	30.0	9.0	2.1	
12.30	3.4	15.0	22.5			
12.45	4.0	12.5	22.5	9.4	3.0	

* The water was retained in the colon for 15 minutes 500 cc. to 550 cc. of water was in the stool, the other remaining in the intestine.

Table 10 shows that the absorption of water from the intestine causes no change in urine ammonia. Nor, according to table 13, does an increase in urine ammonia take place upon absorption of water from the large intestine, as one might think would happen due to the presence of bacterial decomposition.

The absorption of HCl causes an increase in urine ammonia which corroborates the findings of Walter (2) and others. The decrease in urine ammonia upon the absorption of $NaHCO_3$ (table 12) also accords with the observations of others.

These experiments have been repeated upon dogs with duodenal fistula with identical results (table 10a, 11a, 12a). The results obtained are direct evidence confirming the reports of other investigators that urine ammonia is increased by the absorption of acid, and decreased by the absorption of alkalies and not influenced by the absorption of water.

TABLE 14

Chilling the body by exposure to cold. Trial II

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.50–7.20				15	9.1	
7.35	45	20.0	32.5			
7.50	14	32.5	45.0	18	10.0	
8.05	9	32.5	45.0			
8.20	6	25.0	37.5	14	8.4	Began chilling body
8.35	8	22.5	35.0			
8.50	5	25.0	37.5	23	9.2	
9.05	5	22.5	35.0			
9.20	8	17.5	30.0	28	8.8	Stopped chilling body
9.35	5	15.0	25.0			
9.50				15	6.0	
10.20				17	6.2	
10.50				15	6.6	

Diuresis and urine ammonia

Since diuresis accompanied the increase in urine ammonia observed in several of the foregoing experiments, an attempt has been made to see if diuresis in itself might cause an increase in ammonia excretion.

None of the pharmacopeial diuretics proved satisfactory. Upon injection of 20 grains of diuretin, in solution in 20 cc. H_2O into the duodenum, some diuresis resulted with a very marked decrease in urine ammonia. This was due to the sodium salicylate which is said to increase urea synthesis. Injections of water and salt solution intravenously did not prove satisfactory diuretics. It was found that chilling the body by exposure to cold was the best and least troublesome method to produce diuresis.

It is apparent from table 14, as well as from table 10, that the diuresis per se has no important influence upon ammonia excretion. If it did have an influence upon ammonia excretion, one would expect he greatest amount of ammonia to be present during the period of greatest diuresis, which does not generally occur as can be verified by reviewing tables 1 to 10.

TABLE 15

Intravenous injection of water. Data from second injection

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
10.30	43	2.5	12.5			
10.45	7	22.5	35.0			
11.00	4	42.5	50.0	12.8	3.0	
11.15	7	35.0	45.0			
11.30	7	25.0	30.0	20.0	4.7	
11.45	3	22.0	30.0			
12.00	6	22.0	25.0	21.6	6.8	
12.15	7	20.0	25.0			
12.30	3	22.0	30.0	13.4	4.5	
12.45	4	27.0	35.0			
1.00	4	25.0	32.0	11.8	5.1	Injection began at 1.10 and was stopped at 1.29
Inj. 1.15						
1.30	27	22.0	32.0	11.0	5.0	Hemaglobinuria
1.45	10	30.0	40.0			
2.00	7	32.0	42.0	4.4	2.4	
2.15	8	27.0	40.0			
2.30	8	30.0	42.0	4.4	2.4	
2.45	5	32.0	42.0			
3.00	5	30.0	32.0	4.8	2.5	
3.15	4	20.0	25.0			
3.30	8	17.0	25.0	4.2	2.5	
3.45	4	20.0	27.0			Hemaglobinuria stopped at 7.30
4.00	6	25.0	30.0	3.2	2.0	

Intravenous injection of redistilled water: man

It has been reported (unpublished results) by Doctor Sutherland, working in this laboratory, that intravenous injection of water caused an increase in gastric secretion. With this in view and desiring to note if injection of water into the blood stream influenced urine ammonia in any way, three intravenous injections of 200 cc. of redistilled water were made in one man. The injection[4] was made over a period of fifteen minutes, the needle being inserted into the basilic vein. The urine and gastric juice were collected for a period previous to the injection as a control. Table 15 shows the results obtained.

A slight gastric stimulation resulted upon the injection of the water (table 15). The urine amount as well as the urine ammonia was decreased. The acid gastric secretion was not allowed to pass into the intestine and no acid was absorbed, explaining the absence of any increase in urine ammonia. The gastric stimulation was so slight that it is doubtful that there would have been an increase in urine ammonia even if the juice had been absorbed.

GENERAL DISCUSSION

It is well known that the ammonia excretion is markedly dependent upon the balance of acids and bases in the body under physiological as well as pathological conditions. Whether or not there will be an increase or a decrease in urine ammonia during digestion will depend upon this acid-base balance. It must be kept in mind that during digestion, although acid is taken from the blood to form gastric juice, alkali is also used to form pancreatic juice. In fact Hasselbalch's (7) results show almost invariably a greater acidity of the blood, as judged from the H-ion concentration of the urine, for the period after the meal. One would expect the urine to become more alkaline if the blood became much more alkaline during the secretion of gastric juice. Higgins (8), repeating Hasselbalch's work, found "no marked difference between breakfastless and food periods." At any rate urine does not increase in alkalinity during digestion. However, Higgins (8) and Erdt (9) both found an increase in the alveolar CO_2 tension, the former assigning the increase to the slight change in condition rather than to any effect upon blood alkali, and the latter to an increase in the reserve alkali of the blood caused by the secretion of HCl. D. D. Van Slyke, Stillman and Cullen (10) found, by taking samples of blood before the meal and from one-half to two hours after the meal, "that the alveolar CO_2 tension rises after a meal" and that "plasma carbonate in some cases increases slightly, in others does not," results which, Van Slyke says, confirm Higgin's rather than Erdt's explanation of the increased alveolar CO_2 tension during digestion. These findings offer an explanation, then, for the variations in normal and pathological individuals and probably explain why in some of the subjects of my series no increase in urine ammonia occurred during digestion and absorption, for the greater the alkaline reserve or base balance of the blood, the less will be the urine ammonia, and vice versa, for a smaller or a greater amount of NH_3 would be called upon to act as "buffer" to the acid absorbed.

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[4] A hemaglobinuria resulted upon injection and lasted five hours. It was apparent at the first urination and was most marked three-fourths of an hour after the injection ceased. The hemaglobin index was reduced from 92 to 84 (Haldane—Sahli method). The conductivity of the blood was increased slightly. No other reaction was observed.

Since there is no marked change in the acid-base balance of the blood during digestion, it is apparent that the absorption of acid chyme would be followed by an increase in urine ammonia and that any factor that would increase the amount of acid in the chyme or increase the rate of absorption of the acid would be followed by an increase in urine ammonia. On the other hand, if this acid is neutralized before being absorbed, no increase in urine ammonia will occur. Whether neutralization of the acid outside the intestine by $NaHCO_3$, as was done in this study, is comparable to neutralization by the alkaline pancreatic juice in the intestine is a question. Nevertheless it seems obvious that neutralization of the acid chyme by the bases of the alkaline digestive juices, coupled with relatively slow absorption, would not place an extra task upon the acid-base balance of the blood and as a result no increase, or possibly a slight increase, in the urine ammonia would occur. (The diminution in ammonia after the injection of the neutral chyme in table 8 was probably due to the excess in alkalinity of the neutral chyme plus the alkaline digestive juices.) But if the acid chyme is thrown into the intestine at a faster rate,[5] and if its acidity and rate of absorption are increased as when the water-ingestion with the meals is increased, it is obvious that a noticeable increase in urine ammonia would occur for this sudden excess of acid would require ammonia for its neutralization in order to prevent the utilization of the plasma alkaline reserve or a change in the H-ion concentration of the blood.

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[5] See table 1 and a later paper.

CONCLUSIONS

1. The ammonia excretion after the ingestion of a meal varies slightly in the same individual and markedly in different individuals. There was an increase in urine ammonia after the ingestion of a meal in the majority of cases studied in this series. A marked increase occurred in the dogs worked upon.

2. During gastric stimulation by food or by water followed by absorption in the intestine there is an increase in urine ammonia.

3. The degree of increase in urine ammonia upon the absorption of acid chyme is dependent upon the rate of absorption of the acid chyme or, in other words, its fluid consistency.

4. During gastric secretion not followed by absorption in the intestine no increase in urine ammonia occurs.

5 *a*. The absorption of water from the intestine (distal or proximal) causes some diuresis but no change in urine ammonia.

b. The absorption of alkali from the intestine causes diuresis with a marked decrease in urine ammonia.

c. The absorption of acid from the intestine causes some diuresis with an increase in urine ammonia.

6. Diuresis per se causes no change in urine ammonia.

7. Intravenous injection of water causes some gastric stimulation but no increase in urine ammonia or urine output.

So gastric secretion and urine ammonia are related in that the urine ammonia is increased by the absorption in the intestine of the acid product of gastric secretion, provided that this acid secretion is absorbed before neutralization occurs, i.e., at a relatively fast rate.

The writer desires to acknowledge his indebtedness to Doctors Luckhardt and Carlson for their valuable criticisms.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

VII. Venous Discharge from the Adrenal Glands

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As has been pointed out before, adrenin possesses the selective action of stimulating the terminations of the sympathetic nervous system (1). Intravenous injections of adrenin then should duplicate the results of artificial stimulation of the sympathetics to a given tissue.

Of all the endocrine glands, the adrenals and their innervation have probably received the greatest attention at the hands of investigators. Jacobi (2) early described branches of the splanchnic nerves which pass into the adrenal glands and Dogiel (3) later established the anatomical relation of these nerves to the adrenals. The observation by Biedl (4) that stimulation of the splanchnic nerves produced vasodilatation in the gland was soon confirmed by Dryer (5), Tschboksaroff (6) and later by many other observers. The conclusion was that the splanchnics carry vasodilator fibers to the adrenal glands. G. von Anrep (7) in his study of the relations of the suprarenal glands to the normal vascular reactions of the body, observed dilatation to occur in the glands following the administration of adrenin by vein. Neuman (8) using massive doses of adrenin, observed that the blood flow through the adrenal glands was slightly increased during the pressor effect of an adrenin injection.

In the studies of the effect of adrenin on the circulation in the adrenal glands, short lasting injections of varying dosage only have been used. No records of infusions, which probably most nearly simulate the normal discharge of the glands, could be found. It was considered therefore that a careful study of both injections and infusions of varying dosage might conceivably give different results than those recorded.

METHOD

The method of investigation used in this study has been that described for the outflow in previous papers of the series (1). The left adrenal gland was exposed through median and lateral incisions in the abdominal wall and its peritoneal covering removed. The large vein from the abdominal wall which crosses the gland and pours into the adrenal vein was ligated about one inch distal to the gland. An oiled cannula was then inserted into the vein central to the ligature. The adrenal vein was then tied off close to the renal vein. The venous circulation in the short section of vein was thus reversed. This method was used simply for convenience and it interfered in no way with the normal venous discharge from the gland.

Dogs under ether anesthesia were used as experimental animals. The blood pressure was recorded from the femoral artery as before described (1).

RESULTS

The effects of adrenin ("adrenalin") in pressor, depressor and neutral injections and infusions were studied, the drug being injected into the femoral vein.

It was found that the effect of adrenin on the circulation in the adrenal glands was much less than the recorded literature on the subject would lead one to expect. Figure 1 shows the result of a pressor infusion. The only noticeable effect was a short lasting increase in the outflow which occurred during the early part of the rise in arterial pressure. This may probably be explained as due to the propulsive effect of the increased output from the heart. Its brevity strongly suggests that it is not due to a direct action of the drug on the gland vessels.

Figure 2 shows the effect of an injection which is at first pressor and then depressor. During the early part of the pressor stage there occurs the characteristic increase in outflow. During the depressor stage there is a diminution in the outflow. This, however, is only an apparent dilatation in the gland followed by constriction. On closer analysis the change in the outflow can be interpreted as entirely passive. During the whole of the blood pressure reaction the average rate of outflow was one drop per second. This is exactly the average rate of outflow before and after the injection. Regardless of the injection the rate of flow through the gland per minute was the same.

The action then of adrenin on the circulation in the adrenal glands is purely passive.

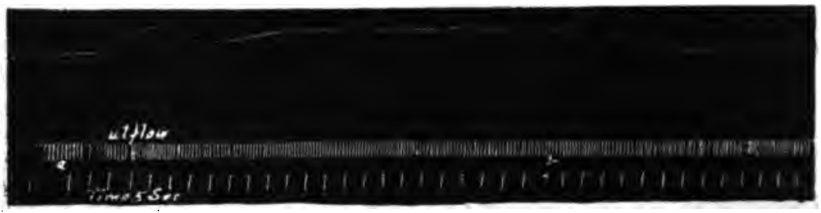

Fig. 1. Effects of an infusion of 20 cc. of 1–100,000 adrenin in two minutes and five seconds on the outflow from the suprarenal vein. Blood pressure manometer slightly damped. Dog weight, 11 kilos.

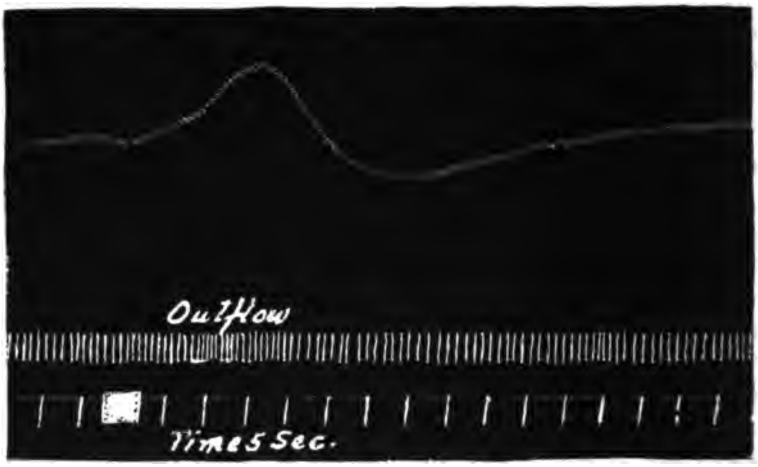

Fig. 2. Effects of 3 cc. of 1–100,000 adrenin on the outflow from the suprarenal vein. Blood pressure manometer damped. Dog weight, 10 kilos.

DISCUSSION

The trauma necessary to the experimentation can hardly be responsible for the observed results. The sympathetics pass to the glands along the suprarenal artery. In cannulating the adrenal vein, the arterial supply was carefully preserved. The failure of adrenin to produce any active change in the blood flow through the adrenal glands leads to the conclusion that the splanchnic nerves do not carry vasomotor fibers to the glands.

Since the completion of this work a paper by Burton-Opitz and Edwards (9) has appeared on the vasomotor supply to the adrenal glands which supports this conclusion. These investigators measured the blood flow through the glands with the stromuhr. They observed no change in the blood flow through the gland from splanchnic stimulation providing the general arterial pressure was maintained at a constant level by simultaneous stimulation of the central end of the splanchnics.

CONCLUSIONS

1. Intravenous injections of adrenin produce no essential changes in the blood flow through the adrenal glands.
2. The changes produced passively follow the general arterial pressure.
3. The splanchnic nerves do not carry vasomotor fibers to the adrenal glands.

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THE ANTIGENIC PROPERTY OF CLOSED INTESTINAL LOOP FLUID

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Whipple, Stone and Bernheim (1), (2), (3), have pointed out the value of isolated closed intestinal loops in the study of some of the obscure features of acute intestinal obstruction. After varying intervals, depending on the location of the closed loop, there accumulates in these loops a substance which, upon injection, causes similar but more intense signs of intoxication than those in dogs with obstruction. The picture is one of severe toxemia with low blood pressure, low temperature, vomiting and diarrhea. It has been stated by these and many other workers that the toxic material in this obstructed portion contains the toxin or toxins of intestinal obstruction and, as a result, a great amount of work has been done to investigate the nature of this fluid. Whipple and his coworkers have concluded that the important toxic factor is of a proteose nature and have endeavored to show that it can provoke its specific antibodies on injection as well as increase the resistance of a normal dog to experimental obstruction. In a more extensive work (4) one of us, working with Doctor Moorhead, failed to corroborate this. The present study is in part an outgrowth of this work, being a further investigation of the problem on an animal much more suited for immunological study than the dog.

A study such as this is somewhat illogical at a time when so little is known of the chemical substances, which must surely be many and varied, present in the closed loop fluid. Inasmuch however as a study of this kind will throw light on this question of the chemical nature of the toxins of the loop fluid, it is justifiable and will be followed soon by comprehensive chemical and analytical studies.

The literature is not very conclusive as to the chemical nature of substances which may act as antigens. In general it is accepted that all proteins have, and all nonproteins do not have, antigenic properties. The question is by no means settled and involves a discussion of the chemical nature of enzymes, the structure of animal and vegetable toxins, etc. See the discussions by Wells (5) and Pick (6). The best established exception is that of Ford's work on mushrooms and poison ivy in which he contends that the poisons are glucosides and nevertheless yield antibodies. Most other works err either in not having the antigen protein free or in confusing an inhibiting substance in the serum with a new formed antibody.

In addition to possible practical therapeutic application, the production of a specific immune body to the toxin or toxins present in acute intestinal obstruction would indicate that these toxins are probably proteins or the closely allied albumoses or proteoses; whereas a failure to produce any sort of immunological reaction after repeated injection with suitable doses in suitable hosts may be taken to point to a toxic substance of non-protein nature.

METHOD

The loop fluid was obtained from intestinal loops produced in the manner described by Dragstedt, Moorhead and Burcky (8). Isolated closed loops of the duodenum, duodeno-jejunum, or beginning jejunum, produced in this manner were found to contain after forty-eight hours from 80 to 100 cc. of a bloody, foul-smelling fluid. This fluid was strained through a coarse mesh to remove the fragments of sloughed mucosa, etc., and then heated for a hour over a water bath at 70°C. This resulted in the formation of a large coagulum which was filtered off. Such treatment was found not to affect the toxicity of the loop fluid. The filtrate was then kept between toluol and chloroform at room temperature. Before using, the preservatives were removed by further heating to 70°C. and the fluid again filtered. Injections were made into the marginal ear vein of healthy rabbits primarily at intervals of four days, later at varied intervals according to the progress of the animal.

The immunological work consisted of *a*, a study of the comparative resistance of normal rabbits and rabbits repeatedly injected with loop fluid, both as to the lethal dose and to the effect of equal doses on the blood pressure; *b*, a study of the serum of injected rabbits with reference to the appearance of any neutralizing or antitoxic substance; and *c*, observations regarding any indication of anaphylaxis.

a. Question of increased resistance after loop fluid injection. Two series of experiments were conducted with reference to this point.

In the first series the rabbits were injected with a dose slightly less than the lethal. This should result in a marked and rapid production of antibodies and the increased resistance could be easily studied by but slight variations in the amount injected; e.g., a rabbit that had received several injections of 2 cc. per kilo should easily resist a dose of say 2.5 cc. per kilo if there was any increased resistance. The fluid used was standardized by injection into five normal rabbits (see table 1).

TABLE 1

Standardization of fluid XO_2

RABBIT NUMBER	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
	grams	*cc.*		
2	1900	7.5	4.0	Dead in 6 hours
3	2800	7.8	2.8	Dead in 12 hours
4	2280	6.8	3.0	Dead in 4 hours
6	2160	4.3	2.0	Dead in 4½ hours
1	1440	4.3	3.0	Survived

It will be observed that there is a very considerable normal variation in the dose that is lethal to rabbits. This of course must be taken into consideration when interpreting results. Two rabbits were then selected and injected for a number of days with fairly large doses. The details of dosage and result are tabulated (see table 2).

Neither rabbit showed an increased but rather a decreased tolerance to subsequent injections. This result was not in the nature of an anaphylactic reaction or protein sensitization phenomenon although, as will be described elsewhere, the injection of a lethal dose of loop fluid results in a post-mortem picture simulating that of an anaphylactic death, the effect being very much like that obtained by Dale and Laidlaw (9) with the depressor substance B-iminazolylethylamin when injected into rodents.

The injection of such large doses resulted in so marked a loss of body weight that although the animals were apparently in good condition upon recovery from each injection, there may have been a greater loss in normal resistance than increase in specific resistance (if there can be any differentiation between the two) to the toxins of the loop fluid. The second series of rabbits were therefore injected with smaller doses over a longer period of time, with more than usual effort to keep them well nourished and in good condition.

As the loop fluid used in this series was different from that of the first series it was standardized by injection into five rabbits with the results as tabulated in table 3. Three rabbits were then injected for a number of days, the total number of immunizing doses being six. The seventh dose was a larger one designed to test the rabbits' resistance for comparison with the normal rabbits in table 3. The procedure and result for each rabbit are detailed in table 4.

TABLE 2

Repeated injection of fluid XO$_2$

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
		grams	*cc.*		
1	1	1440	4.3	3.0	Moderate depression. Recovery
	4	1340	4.0	3.0	Moderate depression. Recovery
	7	1300	3.4	2.6	Slight depression. Recovery
	11	1300	3.9	3.0	Dead in 8 hours
7	1	1650	4.0	2.5	Marked depression. Recovery
	4	1550	3.1	2.0	Slight depression. Recovery
	6	1500	3.0	2.0	Slight depression. Recovery
	11	1500	3.0	2.0	Slight depression. Recovery
	14	1520	3.3	2.2	Dead in 8 hours

TABLE 3

Standardization of fluid I

RABBIT NUMBER	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
	grams	*cc.*		
17	2100	1.0	0.5	No depression
14	1370	1.0	0.7	Moderate depression. Recovery
16	1420	3.6	2.5	Moderate depression. Recovery
18	1500	3.0	2.0	Dead in 22 hours
19	1300	3.9	3.0	Dead in 18 hours

Here again the results indicate no increased resistance. The survival of rabbit 12 is well within the limit of normal variation as illustrated in tables 3 and 1.

The final experiment to test the possibility of an increased resistance in the "immunized" animal was a blood pressure experiment, comparing the effect of equal doses of loop fluid upon the blood pressure in a normal rabbit with that upon the blood pressure of a rabbit repeatedly injected with loop fluid. Several experiments of this kind were done and while the results varied sometimes widely, there were never any variations that could be ascribed to an immunity or lack of immunity per se.

b. The question of a neutralizing substance in the serum of rabbits repeatedly injected with loop fluid. Although the normal variation in resistance to loop fluid is very large in different rabbits (see tables 1 and 3) and it is therefore difficult to establish a definite minimum lethal dose, indicative results should surely be obtained if there is any definite neutralizing substance in the serum of rabbits repeatedly injected with loop fluid. Whipple, Stone and Bernheim (10) report negative results combining the serum from a dog that had been repeatedly in-

TABLE 4

Repeated injection of fluid I

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
		grams	*cc.*		
11	1	1625	1.6	1.0	Slight depression. Recovery
	6	1600	1.6	1.0	Slight depression. Recovery
	13	1470	1.0	0.7	Slight depression. Recovery
	21	1300	0.6	0.5	Slight depression. Recovery
	31	1400	0.8	0.58	Slight depression. Recovery
	42	1500	1.0	0.7	Slight depression. Recovery
	55	1800	3.6	2.0	Dead in 24 hours
13	1	1100	1.1	1.0	Slight depression. Recovery
	7	1020	1.0	1.0	Slight depression. Recovery
	13	1080	0.7	0.7	Slight depression. Recovery
	20	1000	0.6	0.6	Slight depression. Recovery
	31	1180	0.8	0.7	Slight depression. Recovery
	42	1100	1.0	1.0	Slight depression. Recovery
	53	1200	3.0	2.5	Dead in 24 hours
12	1	1360	1.4	1.0	Slight depression. Recovery
	7	1240	1.0	0.8	Slight depression. Recovery
	13	1320	1.0	0.7	Slight depression. Recovery
	20	1140	0.6	0.5	Slight depression. Recovery
	31	1220	0.7	0.6	Slight depression. Recovery
	67	1440	1.0	0.7	Slight depression. Recovery
	83	1600	4.0	2.5	Marked depression. Recovery

jected with large doses of loop fluid, with loop fluid of known toxicity and injecting the mixture. They however report that organ extracts and emulsions (liver, spleen, lung) of "immune" dogs rapidly destroy the loop poison during incubation in vitro. They conceive of an immunity residing in the tissue cells. This finding may be in accord with that of Kraus and Lipschutz (11), who report that the extracts of normal organs are richer in antitoxin against certain bacteriolysins than is the serum of the same animal. This point will be discussed later.

A rabbit that had been repeatedly injected with loop fluid (see table 5) was bled to death, the blood defibrinated, centrifuged and a clear serum obtained. Loop fluid "I" was used which was standardized so that 2 cc. per kilo was the approximate minimum lethal dose (see table 3). Two normal rabbits were injected, one with a mixture of a loop fluid and serum (equal parts) that had been kept two hours in the incubator at 38°C., the other with a mixture that had remained two hours in the incubator and then kept over night in the ice box. Both animals died in less than twenty-four hours with symptoms and post-mortem findings the same as when no serum had been used (see table 6).

TABLE 5

Procedure with rabbit whose serum was tested

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	REMARKS
		grams	*cc.*	
9	1	1800	0.9	Slight depression. Recovery
	7	1600	1.0	Slight depression. Recovery
	14	1490	1.2	Slight depression. Recovery
	22	1340	0.5	Slight depression. Recovery
	32	1420	0.7	Slight depression. Recovery
	43	1400	1.0	Slight depression. Recovery
	61	1400		Bled to death

A similar experiment was done on the guinea pig. The injection of about 1 cc. of loop fluid intraperitoneally into a 350 gram guinea pig caused death in twelve to fourteen hours with symptoms like those in anaphylactic shock, respiratory difficulty, etc. Combining the loop fluid with the serum obtained above in no way altered either the amount necessary to cause death or the symptoms and autopsy findings.

A further test was made to see if the serum used above could destroy the depressor effect of loop fluid on the blood pressure of a dog. The serum was incubated with the loop fluid for two hours and the mixture then kept over night in the ice box. In several such experiments no effect of either neutralization or augmentation of the depressor effect could be noticed.

c. *Observations regarding anaphylaxis.* During the course of the experiments no indications of anaphylaxis were observed. This was the case in the series of rabbits that were repeatedly injected for the experiments above and also in three other rabbits that received sensitizing doses of 0.25 cc. loop fluid and test doses of 1 cc., eleven days later. The symptoms after the injection of large doses of loop fluid into rodents are very much like those seen in anaphylactic shock, however. This is also recorded as the result of the injection of B-iminazolylethylamine by Dale and Laidlaw (9). This substance Barger and Dale have shown to be present in intestinal mucosa (12), an observation which indicates that this interesting substance may be the important constituent of closed intestinal loop fluid. Experiments are at present being done to further compare the physiological action of loop fluid with that of B-iminazolylethylamine.

TABLE 6

Injection of mixture of rabbit's serum and loop fluid

RABBIT NUMBER	WEIGHT	CUBIC CENTIMETERS LOOP FLUID	CUBIC CENTIMETERS SERUM	TREATMENT OF MIXTURE	RESULT
	grams				
20	2000	4	4	2 hours at 37°C.	Dead in 22 hours
21	1450	2.5	2.5	2 hours at 37°C., overnight in ice box	Dead in 18 hours

The absence of any anaphylactic phenomena indicates that we are not dealing with toxic substances of protein or proteose nature. (See Gay (13) and Wells and Osborne (14).)

No test tube experiments, complement fixation or serum reactions, etc., were performed because here no helpful conclusions could be drawn from such observations. If there were no visible reactions upon adding "immune" serum to the test loop fluid it would not indicate that there was neutralization or interaction between the two as a visible reaction is not a sine qua non to such interaction. On the other hand if there were phenomena of precipitation, etc., we could not conclude that there was a reaction between the "immune" serum and the toxic principle as the latter is present in such a composite fluid that any number of results are possible.

DISCUSSION

At no time during the course of these experiments was there any indication of a definite immunity being established in rabbits following the repeated injection of closed intestinal loop fluid. The very large variation in resistance to the injection of this toxic material that is met with in normal rabbits can adequately account for all seeming instances of immunity in our opinion.

The experiments of Whipple and his coworkers in "autolyzing" loop fluid with organ extracts and observing a decrease in toxicity as a result cannot be considered as demonstrating an immune reaction. Ewins and Laidlaw (15) have found that p-hydro-xyphenylethylamine is readily converted into p-hydro-xyphenylacetic acid by the perfused rabbits liver and also to some extent by the perfused isolated uterus, while if the isolated heart is perfused the amine is completely destroyed. They also demonstrated that indolethylamin is converted to indoleacetic acid by the perfused liver (16). More recently Guggenheim and Loffler (17) have made a more comprehensive study of the fate of proteogenic amines in the body. They corroborated the work of Ewins and Laidlaw and demonstrated that a large number of the very toxic amines are rapidly detoxicated after introduction either orally or intravenously. It was demonstrated by perfusion of the liver that this organ can detoxicate these substances by deaminisation and oxidation of the amine to the corresponding carboxylic acid. It is very likely that this detoxication is in part a general tissue reaction and is the phenomenon responsible for the loss of toxicity observed by Whipple et al after incubating the toxic loop fluid with organ extracts.

Davis and Stone (18) have recently shown that normal intestinal secretion is non-toxic upon intravenous injection. This was to be expected inasmuch as dogs show no intoxication after the production of open intestinal loops that are permitted to drain into the abdominal cavity (4), (9). Such secretion, however, when kept free from preservatives and unheated, rapidly becomes toxic producing the same effects upon intravenous injection as closed loop fluid. A rapid and profuse growth of bacteria in this secretion was noted, and while they do not consider it conclusively demonstrated that bacteria are responsible for the development of this toxicity, it is certain that the end products of bacterial activity are concerned.

CONCLUSIONS

1. There are no specific antibodies produced following the repeated intravenous injection of closed loop fluid in rabbits.

2. The toxic principles of closed loop fluid are probably not of protein nature.

The authors wish to acknowledge the helpful criticism of Dr. Preston Kyes.

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STUDIES ON THE THROMBOPLASTIC ACTION OF CEPHALIN

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HISTORICAL

A great deal of light has been thrown recently upon the process of coagulation of the blood and the part played by the different blood constituents in this process. All the various theories on the normal blood coagulation have been recently reviewed by Morawitz (1), Whipple (2) and others.

The theory of Howell (3), (4) on the effect of tissue juices in neutralizing the antithrombin content of normal blood has been also reviewed by a number of investigators. It may be worth while to state briefly this theory, so as to keep in mind the different factors which play a part in normal blood coagulation. The normal plasma contains prothrombin and calcium which go to make up thrombin, the ferment which acts upon the fibrinogen of the blood and converts it into fibrin or the normal blood clot; but normal blood contains also an antithrombin which binds the prothrombin and renders it inactive and before the prothrombin can become active, the antithrombin has to be neutralized by thromboplastin derived from different cells. Howell (5) has also isolated the lipoid cephalin from the brain tissues and has shown that this substance has strong thromboplastic properties in neutralizing the antithrombin present in normal blood and thus allowing the blood to clot rapidly. Hammarsten (6), basing his claim on his own work and that of others, stated that only the calcium precipitated out of the blood by means of a soluble oxalate is necessary to transform the fibrin ferment from the inactive into the active form; the second phase of the process of blood clotting, namely the action of thrombin upon the fibrinogen, can take place also in the absence of calcium salts.

Without going into a detailed discussion as to the different theories on blood coagulation and as to which of them has more scientific evidence in explaining this phenomenon, we will find one thing to hold true and that is that a substance can be obtained from different tissues which has a strong thromboplastic action, greatly accelerating the clotting of the blood. This substance, cephalin, has been isolated by Howell, McLean and others from the brain and other tissues of the body. To test the thromboplastic action of cephalin Howell (5) used a peptone plasma obtained by injecting a solution of Witte's peptone into a dog, 0.4 to 0.5 gram to each kilogram of animal weight; the clear plasma obtained by centrifugalizing the blood of the dog was evaporated to dryness; small quantities of the evaporated plasma were rubbed up with physiological salt solution, filtered and used for the test. The thromboplastic substance was obtained by Howell (5) by extracting dried brain tissues with ether, evaporating the ether, then precipitating the cephalin with acetone and washing with alcohol. A solution of this substance will neutralize the action of the antithrombin of the blood. In another paper Howell (4) reviews the different hypotheses as to the nature of the thromboplastic substance extracted from the tissues and furnishes evidence to show that it facilitates the clotting of the blood only in those plasmas in which antithrombin is present and that it acts by neutralizing this antithrombin.

McLean (7) further reports a method of testing the thromboplastic activity of cephalin; it consists in activating the ineffective thrombin present in fresh serum in relatively large amounts. When to eight drops of oxalated plasma three drops of 0.1 per cent cephalin solution in distilled water and three drops of fresh serum are added, a solid clot is produced in about one minute while the control clots only in thirty minutes to two hours. McLean (8) has further demonstrated the fact that cephalin exposed to the atmosphere or even kept in a desiccator over $CaCl_2$ gradually loses its thromboplastic properties, due to the fact that the unsaturated group of cephalin becomes saturated. Saturated or partly saturated cephalin not only will not exert a thromboplastic action, but a solution of it will give an acid reaction and even retard the coagulation of the blood.

Clinical studies by different investigators have shown that cephalin exerts a very strong hemostatic action when applied to wounds; reference can be made here to the work of Hirschfelder (9) and Horwitz (10).

EXPERIMENTAL

The method of preparation of cephalin was the same, with slight modifications, as that used by Howell and McLean for the preparation of their laboratory cephalin. Brains obtained from different animals were cleaned of all the membranes and blood vessels, macerated to a pulp, spread on plates and dried in a current of warm air for twenty-four to forty-eight hours; the dried residue was then extracted with ether for twenty-four hours; the ether solution of cephalin was removed and the residual mass extracted with another quantity of ether for twenty-four hours. Both extracts were filtered and the filtrates evaporated in a current of cold air to a semisolid residue; the latter was precipitated and washed several times with acetone. The precipitate was then redissolved in ether and filtered. The filtrate was evaporated nearly to dryness, then placed in a desiccator over sulfuric acid where the cephalin was freed from the last traces of ether and acetone without exposing it too much to the action of the air in the last process. The cephalin was then placed in amber glass bottles, evacuated and sealed off.

To test the different cephalin preparations and the effect of the environmental conditions upon this thromboplastic substance, the method used by Howell and McLean was followed. Tubes having a flat bottom, three-quarters of an inch in diameter and two inches high, were used throughout the work for all comparative studies; the observations were carried out at room temperature at about 17° to 19°C., except when otherwise stated. Complete invertibility of the clot was taken as the end point of the reaction. The procedure was carried out as follows: the desired amount of plasma (10 drops used in all cases) was first introduced into the thoroughly cleaned tubes using one clean pipette to fill all the tubes in the experiment; enough distilled water was then added with another clean pipette so that all the tubes, after the addition of the other ingredients, should contain the same quantity of fluid. The cephalin (1 per cent except when otherwise stated) was then introduced with a fresh pipette and soon after the serum or the Ca $(OH)_2$, when used. The introduction of the different constituents into the tubes was done as quickly as possible and time taken at the addition of the cephalin and serum or cephalin and Ca $(OH)_2$.

Difficulties were first experienced in obtaining a good supply of easily available plasma free from any large excess of anticoagulant. The dialyzing of the plasma, particularly in the presence of a large excess of oxalate, did not give an absolutely oxalate free plasma. The use of fresh blood drawn from an animal with a paraffined syringe into paraffin coated containers, as suggested by Morawitz and Bierich, was first tried but this could not supply a material easily available at all times.. It was therefore decided to find out just what concentration of sodium oxalate or sodium citrate was necessary to prevent the clotting of the blood, without any large excess of the salt being present in the plasma.

TABLE 1

The effect of the concentration of the anticoagulant upon the clotting of horse blood
Reading taken after 48 hours

ANTICOAGULANT	QUANTITY	CONCENTRATION	CLOT FORMATION
	grams	*per cent*	
Sodium oxalate	0.05	0.01	+
	0.10	0.02	+
	0.15	0.03	+
	0.20	0.04	+
	0.25	0.05	+
	0.30	0.06	−
	0.35	0.07	−
	0.40	0.08	−
	0.50	0.10	−
Sodium citrate	0.50	0.10	+
	1.00	0.20	*
	1.25	0.25	−
	1.50	0.30	−
	1.75	0.35	−
	2.00	0.40	−

* None first day; coagulation begins only after the first 20 to 25 hours.

Five hundred cubic centimeter quantities of horse blood were drawn into sterile bottles containing different quantities of sodium oxalate or sodium citrate and the clotting of the blood noted; the data are presented in table 1.

It is thus seen from the above table that 0.06 per cent of sodium oxalate or 0.2 to 0.25 per cent of sodium citrate is necessary to prevent the clotting of the blood and keep it unclotted for at least several days, particularly when the plasma is kept in the ice chest, thus supplying an easily available material without any of the difficulties experienced in the process of dialyzing the blood or the peptone bleeding, as used by Howell, and not introducing a large excess of anticoagulant.

By comparing the oxalated and the citrated plasma for the testing of the thromboplastic activities of the cephalin, the results obtained with citrated plasma were more definite and the action of the cephalin more pronounced, due to the fact that the excess of citrate still present in the plasma was more easily neutralized by the calcium introduced than the excess of the oxalate.

To standardize the method still further and perhaps throw some light upon the nature of the action of cephalin, it was decided to substitute in place of a fresh normal serum a standard solution of calcium hydroxide. As was shown above, some authors claim that the thromboplastic action of cephalin consists in activating the prothrombin present in the fresh serum and in the plasma. But in the absence of any fresh serum, the action of cephalin will have to be directed only toward neutralizing the antithrombin or inactivating the prothrombin of the plasma alone, if a sufficient amount of calcium is present to neutralize the excess of anticoagulant Hurwitz (10) emphasizes the fact that only fresh serum should be used; on standing a few days the thrombin of the serum is converted into an inactive form—metathrombin—so that old serum contains less thrombin and more antithrombin. Rich (11) has recently shown that metathrombin is a thrombin-antithrombin compound and is readily formed in solutions containing both thrombin and antithrombin.

TABLE 2

The use of freshly drawn blood

SHEEP BLOOD	CONCENTRATION OF CEPHALIN, 2 DROPS	CLOT FORMATION
	per cent	*minutes*
10 drops	0.2	8
	0.02	14
	0.01	27
	0.002	26
	Water	43

The whole blood was used only in a few preliminary experiments, one of which is reported in table 2, and since the plasma containing the citrate or oxalate was found to be preferable, the latter has been used in all the other experiments.

The effect of the thromboplastic substance is well shown in the above table; even as low a concentration as 0.002 per cent stimulated the coagulation of the blood; and increase in concentration resulted in an increase in the rapidity of coagulation.

TABLE 3

The influence of the amount of anticoagulant present in the plasma upon the action of cephalin

	ANTICOAGULANT	CEPHALIN FROM PIG'S BRAINS, 5 DROPS		
		Fresh horse serum	Clot formation	
			Control	Cephalin
	per cent	*drops*	*minutes*	*minutes*
Sodium citrate..................	0.2	3	240	5
	0.2	6	80	4
	0.2	10	14	3
	0.3	3	720	13
	0.3	6	120	8
	0.3	10	16	4
	0.4	6	240	45
	0.4	10	60	15
	0.5	6	More than 48 hours	120
	0.5	10	Imperfect clot in 24 hours	40
Sodium oxalate..................	0.07	3	24 hours	9
	0.07	6	15 hours	6
	0.07	10	5 hours	5
	0.1	3	Imperfect clot in 23 hours	48
	0.1	10	8 hours, 20 minutes	22

The results presented in table 3 clearly demonstrate the fact that the concentration of the anticoagulant has a decided effect upon the action of the cephalin, as a larger amount of serum has to be added to compensate for the presence of the excess of anticoagulant. This would tend to indicate that the addition of serum is not only for the purpose of supplying an inactive thrombin to the plasma-cephalin mixture but also in supplying a substance which neutralizes the anticoagulant.

To obtain further information on the possible rôle of the serum in neutralizing the anticoagulant of the plasma, a saturated solution of calcium hydroxide was substituted in place of serum.

The relative quantities of anticoagulant and calcium are found to have an important bearing upon the rapidity of coagulation. This confirms the observations made in table 3 that the calcium part of the serum neutralizes the anticoagulant, but this seems to be not the only function of the serum: while an increase in concentration of the serum always results in a more rapid coagulation, the same thing does not hold true with the calcium hydroxide. A further increase in concentration of calcium hydroxide, above the optimum, results in a delay in the rapidity of coagulation; this bears out the observations of Addis (12)

TABLE 4

*The neutralization of the excess of anticoagulant in plasma by calcium hydroxide**

	ANTICOAGULANT	$Ca(OH)_2$	CEPHALIN FROM OX'S BRAIN, 5 DROPS	
			Clot formed in minutes	
			Control	Cephalin
	per cent	*drops*		
Sodium citrate	0.20	3	30	6
	0.25	3	74	9
	0.27	3	170	29
	0.30	3	360	48
	0.35	3	430	52
Sodium oxalate	0.06	3	360	20
	0.07	3	380	30
	0.08	3	Over four days	75
Sodium citrate	0.2	1	62	8
	0.2	2	44	7
	0.2	3	26	6
	0.2	4	37	7
	0.2	5	72	7½
	0.2	6	117	8
	0.2	7		9
	0.2	8		18

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* The calcium hydroxide solution used in this as well as in the following experiments was obtained by shaking some C. P. calcium hydrate with distilled water for five minutes, and then filtering.

and Morawitz (13) that an excess of calcium may delay the coagulation of the blood. The more rapid coagulation resulting from an increase in the concentration of the serum may be therefore explained by the assumption that the calcium optimum has not been yet introduced with the smaller concentration of the serum or, what seems to be more probable, an increased amount of serum will introduce a larger amount of prothrombin which, after it has been activated by the cephalin or after the antithrombin has been neutralized by the cephalin, is converted into thrombin, which will act upon the fibrinogen of the plasma.

TABLE 5

The effect of the concentration of cephalin in accelerating the clotting of blood plasma

CONCENTRATION OF CEPHALIN IN WATER	CLOT FORMED IN MINUTES		DROPS OF 1 PER CENT ETHER SOLUTION	CLOT FORMED IN MINUTES SERUM USED
	Serum	$Ca(OH)_2$		
per cent				
1.0	10	10	1	10
0.8	8	9	2	12
0.6	10½	9	3	13
0.5	8½	11	4	10
0.4	9	11	5	10
0.3	9½	11½	6	13
0.25	12	12	7	12
0.20	13	13	8	12
0.15	12	14	9	12
0.10	15	14	10	10
0.05	18	15		
Control	280	180	Control	230

It was next thought advisable to study the effect of the concentration of cephalin upon its thromboplastic activities. A fresh lot of cephalin prepared from ox brain was dissolved in water so as to make up different concentrations. Horse blood plasma containing 0.2 per cent sodium citrate was distributed in tubes in the usual manner, five drops of fresh horse serum and five drops of the different concentrations of cephalin were added to these; to another series of tubes three drops of calcium hydroxide solution were added in place of the serum; to a third set of tubes a 1 per cent ether solution of cephalin was added.

Several interesting observations can be made from the above table. First of all the amount of a 1 per cent ether solution of cephalin does not seem to play any appreciable rôle in the rapidity of coagulation.

It looks as if enough cephalin has been introduced with one drop of the particular ether solution to produce the coagulation; the further increase may therefore not have any appreciable effect upon the rapidity of coagulation or may even retard it, perhaps by keeping the cephalin in the ether solution and not allowing it to act upon the mixture of serum and plasma. In the case of the water solution of cephalin there does not seem to be any difference between a 1.0 per cent solution and a 0.3 per cent solution, while in the case of the calcium hydroxide the rapidity of coagulation of mixtures to which five drops of a 1.0 per cent and a 0.2 per cent cephalin solution were added was the same; the further dilution gave in both cases a delayed coagulation. The conclusion made from this experiment would be that the concentration of the cephalin is not of great importance in the process of coagulation, if it does not fall below a certain concentration. Above that an increase in the concentration of the cephalin will not result in any further increase in the rapidity of coagulation, while a decrease in concentration will result in a decrease in the rapidity of coagulation. This concentration seems to fall between 0.25 and 0.30 per cent of cephalin solution in water of the particular lots tested when five drops of the solution and ten drops of plasma were used.

TABLE 6

The effect of concentration of cephalin upon the rapidity of clotting of plasma

Ten drops of plasma containing 0.25 per cent sodium citrate; 3 drops calcium hydroxide solution, 1 per cent cephalin solution.

Cephalin drops	0	1	2	3	4	5	6	7	8	9	10
Distilled water drops	10	9	8	7	6	5	4	3	2	1	0
Clot formation in minutes	80	25	18	15	14	10	9	9	10	10	9

TABLE 7

The effect of concentration of cephalin upon the rapidity of clotting of plasma

Ten drops of plasma containing 0.25 per cent sodium citrate, 5 drops of fresh horse serum; 5 drops of fresh cephalin solution

	CONCENTRATION OF CEPHALIN											
Per cent	1	0.5	0.25	0.20	0.15	0.10	0.05	0.04	0.03	0.02	0.01	0.00
Clot formation in minutes	3	4	4	6	7	9	12	16	18	22	45	75

To obtain further information on this optimum concentration of cephalin and also on the time-concentration curve, the same experiment was repeated using lower dilutions of cephalin than in table 5. The data obtained by the use of an old lot of cephalin and three drops of calcium hydroxide are given in table 6; the use of very low dilutions and a fresh lot of cephalin are given in table 7.

The data presented in tables 6 and 7 confirm the previous observations. The rapidity of clotting does not decrease appreciably down to a concentration of 0.25 per cent of cephalin in water; on a further decrease in concentration the time of clotting rapidly increases.

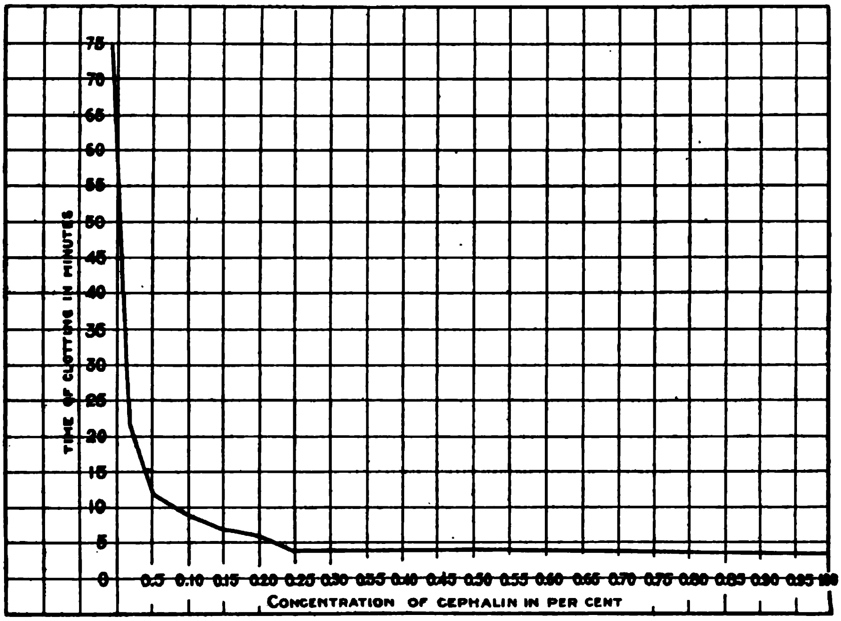

Fig. 1. The effects of concentration of cephalin upon the rapidity of clotting of plasma

Figure 1 shows the relation between the concentration of cephalin and the clotting time of the plasma; the data given in table 7 were used in plotting out the curve.

McLean (8) has shown that the thromboplastic action of cephalin deteriorates with age, particularly on exposure to air and to light, as indicated by its coagulating power and iodine absorption number. These observations were repeated in order to devise a method of keeping the cephalin, especially when prepared in large quantities for the market, with the least deterioration of the material. The advisability of keeping the cephalin in the dark and in vacuated, sealed-off containers at once suggested itself. The vacuated cephalin samples used for this experiment were kept only in a partial vacuum, at about twenty-eight inches obtained by the use of the water pump. The results confirm fully McLean's observations; even an imperfect vacuum proved to be very favorable to the keeping properties of cephalin.

The exposure of the cephalin to the air seems to have an appreciable effect in lowering its thromboplastic properties. The vacuum-kept cephalin was in all instances superior to the lots exposed to the air. No doubt there are slight differences in the thromboplastic properties of the different cephalin preparations even when prepared from the brain of one particular animal, probably due to the fact that since the cephalin is not chemically pure, the impurities and the method of preparation may have a slight bearing upon it The cephalin prepared from the brains of the ox, sheep and pig does not differ greatly in its thromboplastic activities and the differences that do exist may be due to the slight differences in age of the preparations and in the method of keeping, either exposed to the air or evacuated, rather than to their origin.

The observations of McLean (8) are hereby confirmed: cephalin kept in loosely stoppered containers, in the presence of air, will deteriorate but this deterioration will be found by the method used in the previous experiments only when the cephalin is several months old. It was important to find out next how quickly a cephalin water solution will deteriorate.

One per cent solutions of different cephalin preparations were made up and allowed to stand in loosely stoppered glass containers at room temperature. At the end of forty-eight hours and seven days this solution was compared with a freshly prepared solution of the same lot of cephalin, using horse plasma containing 0.25 per cent sodium citrate, 5 drops of fresh horse serum, 3 drops of calcium hydroxide solution and 5 drops of the cephalin solution.

The activity of cephalin seems to diminish rapidly when kept in a water solution for any length of time. This is particularly true with an old lot of cephalin kept under unfavorable conditions. The fact was observed a number of times that fresh cephalin preparations which had a strong thromboplastic power deteriorated only very slowly in solution and often no deterioration was found in solutions forty-eight

TABLE 8

The influence of age of cephalin and method of keeping upon its thromboplastic action

CEPHALIN PREPARATION	FRESH SERUM	CEPHALIN	$Ca(OH)_2$	CLOT FORMATION
	drops	*drops*	*drops*	*minutes*
Ox's brain, fresh	5	5		5
	3	5		6
	2	5		9
	5	1		7
		5	3	8
Ox's brain, 3 months old, kept in vacuo.	5	5		4
	3	5		4
	2	5		4½
	5	1		10
		5	3	4
Ox's brain, 6 months old, exposed	5	5		6
	3	5		8
	2	5		11 imperfect clot
	5	1		12
		5	3	9
Sheep's brain 1 month in desiccator, 2 months in vacuo	5	5		6
	3	5		8
	2	5		8
	5	1		10
		5	3	7
Sheep's brain, 16 months old, exposed to air	5	5		7
	3	5		8
	2	5		9
	5	1		13
		5	3	8
Pig's brain, 2½ months in vacuo	5	5		4
	3	5		5
	2	5		6
	5	1		5
		5	3	5
Control	5			250
	3			Over 12 hours
	2			Over 18 hours
			3	95 minutes

hours old, while in old lots of cephalin which exerted a relatively low thromboplastic action when kept in solution for only twenty-four to forty-eight hours, deterioration set in rapidly. Different cephalin preparations were kept in a water solution for over two weeks and tested every once in a while for their thromboplastic action; it was found that a deterioration set in only in the first few hours, but later the deterioration is less noticeable and, although weaker in their action than the freshly prepared solutions, they still exerted a marked effect in accelerating the coagulation of the blood.

TABLE 9

The influence of age of cephalin solution upon its thromboplastic properties

CEPHALIN PREPARATION	FRESH CEPHALIN SOLUTION		CLOT FORMATION IN MINUTES	CEPHALIN SOLUTION 48 HOURS OLD		CLOT FORMATION IN MINUTES
	Serum	$Ca(OH)_2$		Serum	$Ca(OH)_2$	
Ox's brain fresh	*		5	*		11
Ox's brain fresh		*	8		*	9
Ox's brain vacuated for 3 months	*		4	*		7
Ox's brain vacuated for 3 months		*	4		*	5
Pig's brain vacuated for 2½ months	*		4	*		9
Pig's brain vacuated for 2½ months		*	5		*	8
Ox's brain, 11 months in loosely stoppered container	*		7	*		20 imperfect clot
Control	*		90			
Control		*	72			

All the previous tests were conducted at room temperature. To study the effect of temperature upon the action of cephalin, particularly since its action upon the blood of animals would take place at the temperature of the body, two sets of tests were conducted at room and incubator (37°) temperature.

The effect of a higher temperature is thus found to be very beneficial in the process of coagulation of the blood by the use of cephalin. Since the phenomenon of coagulation, furthered by the introduction of cephalin, whether it consists in the neutralization of the antithrombin or in stimulating the production of thrombin, is an enzyme phenomenon, we would expect that the action will take place more rapidly at a higher temperature.

The question of the nature of cephalin action and the part played by the presence of serum, in addition to the plasma and cephalin, is an interesting one. According to Howell's theory, the cephalin neutralizes the antithrombin thus allowing the calcium to activate the prothrombin and convert it into thrombin which acts upon the fibrinogen of the blood. The Ca $(OH)_2$ added will therefore neutralize the excess of citrate or oxalate present and allow the prothrombin of the plasma which became free to be converted into thrombin and after the cephalin acted upon the antithrombin and neutralized it, to act upon the fibrinogen and convert it into fibrin. The addition of serum to the plasma should therefore increase the amount of available prothrombin and allow the plasma to coagulate more rapidly, but this did not hold true in many cases, as seen in the previous experiments, where the plasma was coagulated almost as rapidly, if not more rapidly, in many instances, when Ca $(OH)_2$ was added in place of serum.

TABLE 10

The effect of temperature upon the thromboplastic action of cephalin

Ten drops of plasma (0.25 per cent sodium citrate), 5 drops of serum or 3 drops of Ca $(OH)_2$ and 2 drops water. 5 drops of fresh cephalin (from ox brain) in different concentrations in water.

	CONCENTRATION OF CEPHALIN						CONTROL	
	0.5 per cent		0.2 per cent		0.1 per cent			
	18°	37°	18°	37°	18°	37°	18°	37°
Serum	4	2	6	4	9	7½	300	180
$Ca(OH)_2$	3½	1¼	5½	4	14	5	78	41

In the following experiments a fresh 1 per cent solution of cephalin was used for the study of the action of cephalin upon plasma, in the absence and in the presence of serum.

The calcium hydroxide seems to act upon the plasma only by neutralizing the excess of citrate, thus allowing the prothrombin in the plasma to be converted into the thrombin which acts upon the plasma and coagulates it; it seems to play no further rôle in the process of coagulation. A further increase in the concentration of the calcium above the amount necessary for the neutralization of the excess of citrate is unnecessary, as is seen from table 12 where, in the presence of three drops of cephalin, three drops of Ca $(OH)_2$ exerted no more action than one drop, two drops of the solution used seem to be enough to just neutralize the excess of citrate and allow the cephalin to act upon the plasma. A further increase in the concentration of the calcium delays the action of the cephalin, as brought out in tables 4 and 12. This deleterious action of the excess of calcium can be neutralized by the addition of serum; the excess of free calcium probably combines with the proteins or salts of the serum, thus allowing the clotting to proceed in a normal manner.

TABLE 11

The influence of cephalin and serum upon the clotting of blood plasma

SERUM	CLOT FORMATION		
	No cephalin	Cephalin 1 drop	Cephalin 5 drops
drops		*minutes*	*minutes*
1	32 hours	25	6
2	26 hours	11	6
3	11 hours	15	5
4	6 hours	10	5
5	1 hour 30 minutes	10	5
6	42 minutes	8	4½
7	38 minutes	8	4½
8	40 minutes	7	3½
9	30 minutes	7	3½
10	18 minutes	7	3
No serum		30	12
No serum	Not clotted in 48 hours		

In adding serum to the plasma we seem to be introducing two different factors, one, probably the action of the calcium of the serum upon the excess of citrate of the plasma, and the other, exerting by itself a thromboplastic action upon the fibrinogen of the plasma, either supplying more prothrombin, which becomes active after the antithrombin has been neutralized and which is converted into thrombin due to the action of cephalin, or perhaps due to some other cause. That the action of serum is due to more than one factor is clearly seen from the fact that, by increasing the amount of serum, we increase the rapidity of coagulation. If the serum acted only by its calcium content there should be a maximum reached, above which a further addition of the serum would be without any effect; but as observed in table 11, the increased addition of serum in the absence of cephalin to the plasma, gradually increased the rapidity of coagulation while, where cephalin has been added, the further increase of serum above a certain concentration does not increase, or only to a very small extent, the rapidity of coagulation. This would indicate that the serum, besides the introduction of calcium, also introduces small amounts of prothrombin, which although the antithrombin has not been neutralized, is able to act in increasing amounts upon the fibrinogen of the plasma. When an excess of cephalin is introduced, enough thrombin is formed or liberated to produce an optimum action upon the fibrinogen, and a

TABLE 12

The influence of cephalin and $Ca(OH)_2$ upon the clotting of blood plasma in the absence and in the presence of serum

$Ca(OH)_2$	SERUM	CEPHALIN	CLOT FORMATION IN MINUTES
drops	*drops*	*drops*	
3		0	120
1		3	4
2		3	3½
3		3	4
3		1	9
3		5	4
0		1	32
0		2	13
0		3	15
0		4	15 imperfect clot
0		5	15 imperfect clot
0		6	10
0		7	7
0	5	1	6
0	5	5	4
0	1	3	7½
1	1	3	5
1	3	3	4
1	5	3	4
2	1	3	3½
2	3	3	3½
2	5	3	3½
3	1	3	3½
3	3	3	3
3	5	3	2¾
8	0	3	18
8	1	3	7
8	5	3	5

further addition of serum above that is necessary for the neutralization of the anticoagulant and the necessary prothrombin or prothrombin-antithrombin compound, will not result in any further acceleration of the clotting.

The addition of cephalin solution to the plasma containing 0.25 per cent sodium citrate, without the addition of any free calcium containing substance, also affects the rapidity of coagulation; so, for example, when one drop of 1 per cent cephalin solution was added to ten drops of plasma, a clot was formed in thirty minutes; when five drops of cephalin were added, a clot was formed in twelve minutes and so on, although in certain instances, as seen in table 12, only an imperfect clot was formed. This would tend to indicate that the cephalin is able to stimulate the clotting of the blood, even without any excess of calcium salts and in the presence of a slight quantity of anticoagulant (sodium citrate); this would be in direct opposition to the well accepted theory of the necessity of calcium salts for the process of coagulation of the blood. The only explanation that could be suggested at present would be that whether due to the presence of cephalin or not, the calcium citrate is ionized in the plasma-cephalin-water mixture and the calcium ions play their part in the process of coagulation, which is therefore accelerated; upon the addition of only a small quantity of calcium the coagulation takes place much more rapidly. The excess of soluble oxalate, the calcium salt of which is much less soluble than the citrate, will not allow this action to take place so readily.

The presence in the mixture of Ca-ions, citrate-ions and prothrombin would act, according to the mass law, in the following manner: in the presence of a large excess of the citrate ions, all the calcium will be precipitated as calcium citrate; in the presence of only a very small excess of citrate ions, the prothrombin of the plasma will then be able to combine with some of the Ca-ions and produce thrombin. This can be represented by the following formulae:

$$\text{Prothrombin excess} \leftarrow \text{Ca ions} \rightarrow \text{Citrate ions excess.}$$

That this holds true is also seen from the behavior of the oxalate in the plasma. When the coagulation of the plasma is prevented by the use of a soluble oxalate, the calcium will be precipitated out to a much greater extent, since the oxalate ions have a much greater affinity for the Ca-ions than do the citrate ions.

The data in table 13 will throw some light upon the nature of the changes that take place in old serum; while three drops of fresh serum will clot ten drops of plasma in eighty-nine minutes and in the presence of two drops of cephalin in nine minutes, three drops of old serum will only clot the plasma in twenty-four hours, but in the presence of cephalin in ten minutes; the same relation holds true with the larger amount of old serum. This deficiency in the old serum can be corrected by the addition of Ca $(OH)_2$, showing that the change that has taken place in the serum might have had something to do with the calcium transformation in the serum. The old serum has no deleterious effect upon the fresh serum, it has even a stimulating effect:

TABLE 13

The action of old serum upon the clotting of the blood

Ten drops of plasma containing 0.25 per cent sodium citrate, horse serum 7 days old, fresh horse serum and $Ca(OH)_2$ solution.

OLD SERUM	FRESH SERUM	$Ca(OH)_2$	CEPHALIN	CLOT FORMATION
drops	*drops*	*drops*	*drops*	
3				24 hours
3			2	10 minutes
3		2		15 minutes
3		2	2	5 minutes
3	3		2	6½ minutes
5	5		2	6½ minutes
5				12 hours
5			2	8 minutes
5		2		23 minutes
		2	2	5 minutes
	3			89 minutes
	3		2	9 minutes
	5			72 minutes
	5		2	7 minutes
		2		60 minutes
		2	2	10 minutes

while three drops of fresh serum, in the presence of cephalin, produced a clot in nine minutes and five drops in seven minutes, upon the addition of three drops of old serum to these the clot was produced in six and one-half minutes. Since, according to Hurwitz, the thrombin is changed in old serum to metathrombin, it is possible that the serum has not become yet old enough for all the thrombin to change into another form.

The presence of both calcium and serum accelerate coagulation; these act in a manner similar to a large amount of serum.

The function of cephalin consists therefore in neutralizing the antithrombin of the normal blood, according to Howell's theory; the increased rapidity of coagulation in the presence of calcium salts or fresh serum is due to the fact that calcium is, as is generally accepted, an important factor in the process of the coagulation of the blood; the action of the serum consists then both in supplying calcium to the plasma-cephalin mixture and in supplying a larger amount of prothrombin.

A detailed study of clinical uses of cephalin will be published elsewhere; a large number of clinical observations on the use of cephalin has accumulated tending to show the great value of cephalin in quickly arresting hemorrhages from bone, kidney, muscle and other tissue surfaces, as well as to the bleeding wounds of hemophiliacs.

TABLE 14

The action of cephalin gauze upon the rapidity of clotting of blood plasma

Clot formation in minutes

TEMPERATURE	VACUATED	EXPOSED	CONTROL GAUZE
20°	17	25	62
37°	7	10	24

In view of the fact that cephalin in water solution rapidly deteriorates and also since it is usually necessary to have it in a sterile form, a method was worked out[1] by which surgical gauze is soaked in an ether solution of cephalin for a certain definite period of time; the ether is allowed to evaporate and the gauze impregnated with cephalin is packed in suitable containers and sterilized. It is not here the place to give the details of the methods and use of this marketable preparation; mention will only be made of the effectivity of this gauze, both kept in a vacuum and in an open container, in coagulating blood.

The same method as outlined above was used; the gauze used in this experiment was kept for about half a minute in a 5 per cent ether solution of cephalin; the gauze was sterilized in the autoclave at fifteen pounds pressure for half an hour. Three cubic centimeters of horse plasma containing 0.25 per cent sodium citrate were introduced into the glass containers, three drops of $Ca(OH)_2$, five drops of distilled water and about one-half square inch of the gauze finely cut were added to that. The data are given in table 14.

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[1] Also suggested by Cecil (14).

It is thus seen that the cephalin-impregnated surgical gauze exerts the same thromboplastic action as the cephalin itself and although in this case the differences are not so striking as in the case of the latter, we can explain it by the fact that cephalin acts in solution and it takes a few minutes before the cephalin from the gauze becomes dissolved in the blood fluid.

SUMMARY

1. In testing the thromboplastic action of cephalin the method of Howell has been adopted with the following modifications:

a. Blood plasma containing 0.2 per cent to 0.25 per cent of sodium citrate was found to be well suited for these tests.

b. A standard solution of Ca $(OH)_2$ can be substituted for serum, thus eliminating one or more unknown factors.

2. The function of the serum in the plasma-serum-cephalin mixture consists in supplying the calcium necessary for neutralizing the excess of anticoagulant in the plasma and probably in supplying more prothrombin.

3. By using Ca $(OH)_2$ to neutralize the excess of anticoagulant in the plasma, an optimum concentration is found, above which the excess of calcium will delay the coagulation of the plasma.

4. There is always a maximum concentration of the cephalin in the water solution which gives the most rapid coagulation; a further increase in the concentration of the cephalin will not result in an increase in the rapidity of coagulation; by decreasing the concentration of the cephalin below this maximum a delay in the coagulation will result. This concentration seems to fall between 0.25 per cent and 0.30 per cent for the lots tested.

5. Cephalin kept in a vacuum acts much better as a thromboplastic agent than the same lot of cephalin kept in loosely stoppered containers.

6. The cephalin obtained from the brains of different animals does not differ greatly as a thromboplastic agent; the slight differences obtained were probably due to the impurities and methods of keeping the material.

7. Cephalin dissolved in water loses to some extent its thromboplastic properties, particularly old lots of cephalin kept exposed to the air.

8. Since coagulation of the blood is an enzymatic phenomenon it is much more rapid both in the presence and in the absence of cephalin at 37° than at room temperature.

9. Serum seven days old is less active than fresh serum in accelerating the clotting of the blood; this was corrected, with the method used, by the addition of a solution of Ca $(OH)_2$; fresh serum is not depreciated in its action upon the plasma-cephalin mixture by the presence of old serum but is even slightly accelerated.

10. Surgical gauze impregnated with cephalin was also tested by the above method and found to increase the rapidity of the coagulation of the plasma.

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THE ACTIVITIES OF DECEREBRATE AND DECEREBELLATE CHICKS

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The investigation reported in part in this paper is an attempt to throw additional light on the nature of the influence exerted by the cerebrum over the lower nervous centers in a form, the domestic fowl, in which the importance of this part of the nervous system is conspicuously less than in man. That birds are relatively little affected by removal of all that part of the cerebrum in front of the optic thalami has long been known (1). The standard picture of the decerebrate pigeon is too familiar to call for detailed repetition here. We need merely recall that locomotor activities, even those so complex as the act of flying, are preserved without demonstrable impairment while functions clearly dependent on associative memory are lost or seriously affected.

In connection with a consideration of the process of acquirement of various complex activities during the early life of the individual, it occurred to us that observations of decerebrate young birds might afford some information concerning the relative parts played by the cerebrum and the lower brain centers therein. This paper deals with the behavior of the forms studied. Subsequent communications will report the results of anatomical studies and observations on vision.

Material. Our observations were made upon young chicks. Of these the first lot, seven in number, were hatched under a hen, February 1 to 3, 1917. The second lot of twenty-nine were incubator hatched, March 1, 1917, as were also the third lot of six, March 31, 1917. All the chicks were of the white leghorn breed. We had no difficulty in maintaining them in good health. Of the entire series only two, both from the first lot, developed any characteristic ailments. These became "droopy" and died on the sixth and seventh days respectively. A feature of the chick that makes it a favorable object for such a study as this is the presence at hatching of a considerable residue of yolk which nourishes the bird during the first two days.

Operative Procedure. The operations performed were as follows:

1. "Standard" decerebration. In this operation we attempted to repeat, so far as possible, the usual procedure for preparing decerebrate pigeons for class demonstrations. A longitudinal skin incision was made along the median line of the skull; then with stout scissors skull and dura were cut through transversely just anterior to the fronto-parietal suture. This cut was about 5 mm. long, approximately half on each side of the midline. From the ends of this first cut other cuts were made forward for about 3 mm. The flap of bone resulting from these cuts was lifted and the tip of a glass tube connecting with an aspirating pump was inserted. We found a glass tube as large as could be passed readily through the opening of the skull (outside diameter of tip 4 mm.) more satisfactory than a smaller tube. By means of this tube the brain substance and blood were sucked out of the cranial cavity. By watching through the opening and taking only that brain substance which came freely, the desired amount was obtained in every case. The hemorrhage was not severe and no fatalities occurred from this operation. Since the operation, to and including removal of the hemispheres, occupied only about five seconds, anesthesia was not employed.

2. "Deep" decerebration. In this procedure we attempted to remove, in addition to the mass of brain substance taken in the "standard" operation, a small additional amount from the thalamic region. After the hemispheres had been removed in the manner described above, the suction tube was thrust into contact with the brain substance still visible on the floor of the cranial cavity and a small mass withdrawn. As variations on this method we tried cutting away a small slice with fine curved scissors or with a sharp bent knife.

3. "Shallow" decerebration. In birds the pallium is very thin and according to certain investigators (2) has less importance in determining the nervous activities of these creatures than the underlying corpora striata. We attempted to remove the pallium and leave intact the corpora striata by making the opening through the skull with great care so as to avoid injuring parts beneath, using a much smaller suction tube (2 mm.) and keeping the tube always parallel to the roof of the skull so that superficial structures might be withdrawn without disturbing deeper ones.

4. Unilateral decerebration. As the name implies, this consisted in the removal of only one or the other hemisphere. Except in the case of chicks just hatched, ether anesthesia was employed during this operation. We obtained unilateral "standard" decerebration and also, in a single case, unilateral "deep" decerebration. The procedure was similar to that described above except that the skin and skull incisions were confined to the side of the midline from which cerebral tissue was to be taken.

5. *Ablation of the cerebellum.* We were successful in one of two trials in removing a considerable portion of the cerebellum of a new hatched chick. This was accomplished by suction through an opening made with a 3 mm. drill through the parietal bone lateral to the midline. The operation was performed without anesthesia. There was more hemorrhage than in standard decerebration but not enough to indicate that the effects observed were due to loss of blood.

Care of the chicks. Since decerebrate birds do not feed themselves successfully nor drink spontaneously a technique for giving the chicks food and drink had to be adopted. After consultation with professional poultry breeders and considerable experimentation we settled upon the following ration. Dry bread was pulverized in a mortar and then thoroughly mixed with hard boiled egg, shell included, in the proportion of two parts bread to one of egg. To this a small amount of charcoal and some finely chopped alfalfa hay were added. This mixture was dried in the sun. When about to be fed a sufficient portion of it was moistened with water to permit it to be formed into small pellets. The chicks were fed by holding the beak open and inserting a pellet into the throat whence it was swallowed as soon as the beak was released. In addition to this food mixture a commercial chick food consisting of mixed grains and seeds chopped fine, was administered, together with a small amount of "grit." The average feeding consisted of 1.5 grams of the food mixture and 1 to 4 grams of grain and grit. The chicks were fed twice daily. In connection with each feeding as much water was given as would be taken without appearance of distress. Usually additional water was given at intervals during the day. A fixed rule was adopted that the crop must be empty at least once daily. If it was found to contain food at any feeding time only water was administered. The amount of food given was controlled by weighing each chick before and after each feeding.

Behavior of young normal chicks. Careful studies of the activities of young chicks have been made by various observers (3), (4), (5), (6). For our purposes it is convenient to attempt to classify the activities described by these investigators into fairly related groups. Four such suggest themselves. *a. Locomotor activities.* By these we mean walking, running, flapping of wings, jumping up into a support or down from one, balancing on a narrow perch, craning the neck downward from a perch and other movements obviously allied to these. *b. Self cleaning activities*, including preening self, scratching self, wiping bill. *c. Feeding activities*. Pecking at moving and stationary objects, seizing and swallowing food, scratching in litter, drinking. *d. Miscellaneous activities apparently of a higher order:* tendency to run to moving objects, including other chicks; tendency to seek solitude when in possession of a large tidbit; tendency to attempt to seize such a tidbit from another chick; the "fear reaction" (see below); the manifestation of "wildness" when approached; acts apparently based on memory such as the deliberate return to the brooder after escaping from it. By the "fear reaction" we mean the very characteristic behavior of young chicks, described by Thorndike (3) and others, in response to strange sounds or objects. We found shrill "trilling" admirably adapted for eliciting this reaction. In our experience this sound was much more effective than the "mew" used by Thorndike. When the stimulus is given the chicks dart rapidly in various directions and then become immobile, remaining so for some time, a minute or more in certain cases. The attitude during immobility is often very striking Usually the chick crouches and holds the head in the air, with neck slightly bent so that one eye is directed toward the sky. Sometimes immobility overtakes the chick in an unusual situation. On one occasion we observed a chick immobile in the act of stepping over the rather high threshold of the warm chamber. He remained motionless with one leg outside and the other inside for fully thirty seconds.

The time of appearance of the various activities as observed by ourselves and reported by other investigators is substantially as follows: The activities of group *a*, locomotor actions, begin to appear very soon after hatching. Most chicks walk and flap their wings on the first day and run, jump, and balance well by the second or third. One gains the impression from watching them that the manifestion of activities of this class waits on the attainment of sufficient muscular strength rather than on any particular stage of brain development. In other words, one is tempted to conclude that the nervous mechanism of locomotion is adequate at the time of hatching but that the muscles do not attain adequacy for some hours thereafter (Thorndike, p. 286).

In group *b* the "preening" reaction is first to appear, being shown by chicks only a few hours old. Scratching of self and wiping bill are occasionally seen on the first day and typically on the second or third.

The activities of group *c* seem to appear in fairly definite order. Most chicks follow and peck at small moving objects, such as the metal tip of a pencil, within twelve hours of hatching; often before walking itself is well performed. Pecking at stationary objects, grains or specks, appears almost as soon in some, a day later in others. A few precocious chicks seize and swallow grains with considerable accuracy on the first day. Most do not do so till the second day and Breed (5) reports improvement in accuracy of seizing up to the third day and attainment of ultimate accuracy by the eleventh day. We did not make quantitative observations on accuracy of seizing but noted that most of our chicks were obtaining enough food on the second day to distend their crops.

We did not observe definite scratching in litter till the third day, although we noted in two instances on the second day what appeared like feeble beginnings of the action. Drinking is learned by most chicks as early as the second day, and chicks will make characteristic movements, lifting head and swallowing, within twelve hours of hatching, if the bill is dipped into water.

Some of the activities of group *d* appear early; others are not seen till a considerable time after hatching. We observed a pronounced "fear reaction" on the second day, and Morgan (6) cites an observer to the effect that a type of fear reaction may occur in certain species of birds even before hatching. The tendency to run toward moving objects is clearly manifest by the third day. The characteristic displays of greediness, snatching at food in other chicks' mouths and attempting to prevent this, we observed first on the twelfth day. "Wildness" is said by Thorndike (loc. cit., p. 288) to develop during the first month, with possible beginnings as early as the tenth day. We have seen it well developed in chicks two weeks old.

The reaction which we mentioned above as suggesting memory, namely the deliberate return (by jumping over a wooden wall one foot high) to the brooder whence escape had been made, we noted first on the sixth day. Thereafter the chicks jumped out and in with great freedom.

Behavior of "standard" decrebrates. For purposes of description these will be placed in series according to age after hatching at which decerebration was performed. The first series includes four chicks decerebrated within an hour or so after hatching, in every case before becoming dry. In the second series are four chicks decerebrated between the third and eighth days. The third series consists of three chicks decerebrated between the tenth and the twenty-sixth days. The first series is in general the most interesting since in its members the cerebral hemispheres may reasonably be supposed not to have commenced functioning actively at the time they were removed. The entire nervous development of these chicks might be said to have occurred, then, independently of cerebral influences.

In general the locomotor activities (group *a*, p. 398) appear to develop in these early decerebrates as rapidly as in normals. The "shock" of operation seems to be very transitory. In two to four hours after it is performed the chicks are in general appearance on a par with normals of the same age. Three of those observed by us walked well before the end of the first day; the fourth stood unsteadily on the first day and walked well on the second. They jumped, flapped their wings, and perched about as early as normals. One jumped out of a chalk box on the third day, another on the fifth, and another on the seventh. We had the impression in the case of those that were slow about performing this feat that absence of stimulation rather than lack of ability was responsible. The decerebrates placed within the chalk box tended at once to show the drowsiness so characteristic of decerebrate birds. This drowsiness is not so persistent in young decerebrates as in older ones and we were always rewarded at one time or another with attempts to escape from the box. Some of the decerebrates were definitely less skilful in this feat than any normals of the same age but the difference did not seem to us sufficiently marked to be undoubtedly significant. The self-cleaning activities (group *b*, p. 399) are also as prompt in appearance in these decerebrates as in normals. They preened themselves and wiped their bills on the first day and scratched themselves on the second.

In the feeding activities (group *c*, p. 399) the first striking differences begin to appear. Pecking at both moving and stationary objects is established by the second day but the actual seizing and swallowing of food, activities which, in normal chicks, follow closely upon the commencement of pecking, fail to progress beyond what might be called the accidental stage. If one of these chicks is placed on a table on which grains are scattered it pecks frequently although rather aimlessly. At rare intervals a grain is taken into the beak. A normal chick, after the third day, frequently mouths grains that are seized and works them back into a position whence they can be swallowed. We have not seen standard decerebrates do this. Apparently any grains that are swallowed are such as in the act of seizing happen to strike far enough back in the mouth to evoke the swallowing reflex.

Scratching on the floor of the brooder or in litter was first seen in a chick of this group on the sixteenth day. In normal chicks as previously noted, scratching is well established by the third day. These decerebrates appear to scratch in much the same manner as do normals, although they show even less discrimination than the latter in the selection of spots where the act might have some degree of utility. They are by no means so persistent in scratching as are normals.

A striking fact with regard to these decerebrates is the complete disappearance in them of spontaneous drinking. If their bills are dipped in water they make appropriate drinking movements, or if they peck accidentally in the drinking vessel they do likewise, but we noted no suggestion of deliberate attempt to obtain water nor did we observe that characteristic "scooping" movement described by Breed (loc. cit., p. 8) as preliminary to and included in the act of drinking. They were often longer without water than the normals but in spite of their presumably greater thirst the reaction did not appear.

Of the miscellaneous reactions (group *d*, p. 399) the most conspicuous in these chicks, decerebrate from hatching, is the tendency to run toward moving objects. This is very striking particularly during the first fortnight. Movement of a bright object in their field of vision will almost always cause them to approach quickly. Whenever any chick runs for any reason the decerebrates run with him. In connection with the "greed" reaction the decerebrates run in close company with the normals that are engaged in pursuing the possessor of a tidbit but we have not seen any of them attempt to snatch the latter, as normals continually do.

The "fear reaction" is either wholly absent from these decerebrates or only its initial manifestation is present. Usually the decerebrates pay no attention to the trilling. We have seen a few instances of apparent reaction to it but only in the form of rapid darting for a few steps. Instead of the subsequent immobilization, seen in normals, these resume promptly their former activities. "Wildness" is wholly absent. These chicks offer no objections to handling or to the approach of the hands. Acts clearly dependent on memory were not observed to occur.

Series 2. The second series of standard decerebrates, those in which the operation was performed between the third and eighth days, showed some interesting contrasts in comparison with the chicks decerebrated at hatching. These chicks before decerebration fed themselves, drank, scratched in litter, showed the "fear reaction" and behaved generally in a manner to indicate that the normal activities were well established.

Our special interest in this series was to observe whether any of the activities which fail to appear or which are slow in appearing in chicks decerebrated from hatching would show themselves, or appear sooner in these, decerebrated after the activities were well developed. Our observation was that the locomotor and self cleaning activities, which develop normally in the early decerebrates, continue unimpaired in these. We noted one feature of locomotion which seems worthy of record. In all the chicks decerebrated after the second day there was a period, starting the day after decerebration and continuing for several days thereafter, in which there was a pronounced tendency to run in straight lines. The chicks would start and run till their course brought them to the wall of the brooder. Thence they would follow along the wall, slowing down abruptly at the corners, turning them skilfully, and proceeding. Certain chicks kept the wall always at their right, others kept it always at their left. This tendency was rendered striking by the circumstance that the inclined approach to the warm chamber of the brooder was so placed that those keeping the wall to the right would always run up it and into the warm chamber. This would be encircled, then the chick would emerge, swing sharply to the right, jump off the edge of the incline and proceed. Those that ran with the wall at the left were never seen to enter the warm chamber in this manner. They would approach the incline from the side, pass along its foot, and continue around the edge of the brooder. This running tendency disappeared after a few days and we are inclined to attribute it to some irritation from the seat of injury.

In connection with this running reaction we observed a typical manifestation of the decerebrate condition. The brooder was so placed that at a certain hour a bright strip of sunlight about 2 cm. wide lay along the floor in the path of chicks running along the wall. Two different chicks were observed repeatedly as they approached this bar of sunlight to jump over it as over a physical obstruction; none of the normals behaved similarly.

The feeding activities in these chicks corresponded in general with those of the early decerebrates with one exception. Whereas none of the latter began to scratch on the floor before the sixteenth day, all those decerebrated between the third and eighth day resumed scratching within eight days. All the decerebrates of this series were scratching at least three days younger than the youngest early decerebrate to begin the act.

The miscellaneous activities of these chicks were the same, so far as our observation extended, as those recorded above for the early decerebrates.

Series 3. The chicks decerebrated after the eighth day were grouped separately from those just discussed because of their different behavior in connection with the feeding activities. Instead of preserving the reaction of scratching unimpaired or resuming it after a short delay, as we rather expected from our observations on the other groups, we were surprised to note that the scratching activity disappeared completely and was not resumed. One of these chicks, decerebrated on the tenth day, lived forty-two days thereafter, showing during that time most of the activities common to decerebrate chicks, but without scratching, at least during the time of observation which included most of the daylight hours. The pecking activity seemed also to be less manifest in the chicks decerebrated later than in those decerebrated young. The chick mentioned above, decerebrated on the tenth day, was not seen to peck at all for six days and after that only at occasional intervals during a month. A chick, decerebrated on the twenty-sixth day, had not been seen to peck up to the time it was killed on the thirty-third day.

In general all the standard decerebrates were more active during the first month of life than later. This appeared to hold irrespective of the age at decerebration. Those decerebrated early showed a long period of activity, while those decerebrated late lapsed quickly into a sluggish state. The appearance of all these decerebrates at the end of a month of life corresponded closely with the usual description of the adult decerebrate pigeon. Occasional intervals of activity, probably associated with hunger, alternated with periods of standing quietly in one spot, apparently sleeping. All these chicks showed the characteristic drooping of the feathers described for decerebrate birds, developing gradually and finally becoming very marked.

"Shallow" decerebrates. In this category were four chicks. Two of these were prepared with great care to keep the injury superficial. In the other two an attempt was made to obtain a condition intermediate between the standard and the shallow decerebration. Of the two designedly "shallow" decerebrates, one was operated on at hatching, the other on the eighteenth day. One of the two intermediates

was prepared at hatching, the other on the fifth day. All are grouped together because in the main their reactions were similar. All fed themselves successfully, drank spontaneously, scratched actively and in general deported themselves like normals. Certain minor features of difference were noted. On the whole these chicks appeared to have less initiative than normals and to be less "wild." One or two of them showed remarkable subservience to external stimuli. For example, repeated tapping on a hard surface sufficed to attract one to the point tapped. By the use of this stimulus this chick could be led all over the laboratory or caused to jump up into a chair and thence to a table and down again. It reacted in this manner on a demonstration table before an audience, a situation in which normal chicks show signs only of fright or bewilderment.

One of the "intermediates" was interesting in that while most of his activities were similar to those of the "shallow" decerebrates, his behavior in scratching resembled that of "standard" decerebrates. This chick was prepared at hatching. He was first seen to scratch on the floor on the twentieth day thereafter. None of these chicks showed signs of operative "shock" in any noteworthy degree. Those prepared some days after hatching resumed their usual activities within three or four hours after operation. Those prepared at hatching appeared to develop as rapidly as normals of the same age.

"Deep" decerebrates. We attempted on six chicks the operation described above as "deep decerebration," namely the mutilation of some portion of the thalamus, in addition to complete removal of the portions anterior thereto.[1] These chicks ranged in age between five and fourteen days. We did not try this operation on any new hatched chicks. Three of the six chicks in this series died within twenty-four hours. The others lived three, five and six days, respectively. Those that died early showed few significant features. A tendency to rigidity (decerebrate?) was noted in two of them immediately following operation. One of them struggled wildly and presented an appearance which would, in a normal chick, suggest suffering—eyes staring and bill open. Another stood and shook its head when disturbed; it remained quietly in one spot except when stimulated.

All the three chicks that lived more than one day showed the swallowing reflex and all stood and walked about. In each case the walking was unsteady and presented an appearance of effort. There was manifest difficulty in maintaining equilibrium together with apparent muscular weakness. The visual reflexes seemed to be present. One chick, No. 22, that lived five days, was seen to preen itself on the first day before it had attempted to stand. On the fourth day it attempted repeatedly, while standing, to preen the feathers on the back near the tail. On four trials it toppled over backward, recovering itself after violent struggling. At a fifth trial it succeeded in making the preening movements without falling.

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[1] From one chick, no. 15, only the right cerebral hemisphere was removed. This chick lived only six days.

Chick 15, from which only one cerebral hemisphere had been removed, behaved in the same general manner as the other deep decerebrates. It showed no activities that were not shown by other deep decerebrates from which both hemispheres had been removed. It was observed to hold its tail toward the uninjured (left) side. Its compensatory tail movements were noted to be slight in comparison with those of normal chicks or of half-decerebrates in which the thalamus was intact.

All these deep decerebrates were much more active,and to judge from appearance, in much better condition when in a warm environment than when placed in a cool spot. The difference was obvious. We did not attempt to determine whether or not there was an actual lowering of body temperature in the cool surroundings although the difference in behavior suggested that such a lowering might occur (8).

Unilateral decerebration. Beside the half decerebration described above, we removed one cerebral hemisphere from each of four chicks, two at hatching and two at two weeks old. In one of each age the left hemisphere was removed, in the other the right. Our aim in these experiments was primarily to observe the effects of "shock" as distinct from those of removal of nerve substance. We were and are of the opinion that surgically the removal of one hemisphere constitutes as pronounced a basis of "shock" as the complete ablation of the cerebrum. The observations of interest on these chicks had to do, for the most part, with the entire absence of disturbances attributable to "shock." None of them showed more than a transitory interruption of function. Except on close examination they were indistinguishable from normals of corresponding age. The most obvious difference between them and normals was in the matter of vision. All the half decerebrates retained normal vision on the side of injury and showed visual reflexes, but not psychic vision, on the side opposite the injury.[2]

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[2] A detailed study of vision in these chicks will be presented in a separate communication.

Ablation of the cerebellum. The single chick in which ablation of the cerebellum was successfully accomplished lived six days thereafter. The following were the chief points noted in respect to behavior: The chick made no attempt to stand; except when held it lay on side or back. It was very active, struggling almost continuously. Its most violent movement consisted in kicking out with both feet together. There was also much flapping of wings. When held in the hand with feet curled up to the body it would remain quiet. Any attempt to put it down led to a renewal of the violent kicking so that it had to be kept closely wrapped to prevent self injury. There was loud and persistent peeping much of the time. The head was thrown back and forth repeatedly. There seemed to be no pronounced loss of strength during the first five days; on the sixth day weakness became pronounced. The movements of the legs appeared to be wholly incoordinate, although the chick was able to make coördinated movements of some parts. Thus when held in the hand, the situation in which it appeared to be most comfortable, the chick made directed movements of head and neck. When the bill was dipped in water a typical drinking reaction occurred. This was seen on the first and second days. It was successful only when not too extensive. If the head happened to be raised too far there succeeded to the drinking movement violent backward and forward motions of the head. On the fifth day the chick preened itself on the wing, pecked at a moving pencil point and made what appeared to be pecking movements at grain placed within easy reach. All these activities occurred while the chick was being held in the hand. So far as we could judge, hearing was normal as well as sight. Careful tests for compensatory movements were made for us by Professor Weymouth. None could be demonstrated although their presence could not be absolutely excluded. In comparison with the conspicuous manifestations of equilibrating power in standard decerebrates, compensation in this chick was certainly negligible.

DISCUSSION

Although comprehensive analysis of the observations described in this paper must wait for the completion of the morphological studies now in progress on the operated brains, there are certain points which seem sufficiently clear to justify comment.

Our observations seem to us to demonstrate that in chicks locomotor and self cleaning activities develop and are mediated in complete independence of the cerebrum. The first definite suggestion of cerebral influence is seen in connection with feeding activities. The distinction suggested by Breed (5) between striking at grains and actually seizing and swallowing them, appears from our work to have a definite anatomical basis. Thus our "standard" decerebrates developed the pecking reaction as far as "striking" almost as early as normals. The further progress of the reaction to the point of successful self feeding, which in normals occurs within twenty-four to forty-eight hours after the initiation of "striking," fails in the decerebrates completely. The suggestion is that the act of "striking" is in a category with locomotion and self-cleaning, whereas "seizing" and "swallowing" belong in a different category and one which is dependent on the cerebrum.

More complex is the analysis of the act of scratching in litter or on the ground. In normal chicks this is established by the third day and continues thereafter throughout the normal life of the individual. In none of our "standard" decerebrates did this activity appear so promptly although it did appear ultimately in all in which decerebration was performed within eight days after hatching. We have here a suggestion of an activity that is not necessarily dependent on the cerebrum but under normal circumstances develops in relationship with it. Our observations suggest, further, that the ability of the lower parts of the nervous system to establish this reaction, independently of the cerebrum, is quite limited. The fact that none of our chicks, decerebrated after the tenth day, reacquired it, indicates a nervous plasticity during the first days that does not persist thereafter. Confirmatory of this idea is our observation of the increasing general sluggishness after the first month. In fact studies of the learning process as a whole in chicks suggest that the period of plasticity is confined to the first few days (Breed, loc. cit.). That young decerebrate kittens are similarly more responsive than adult cats has recently been shown by Weed (7).

In sharp contrast with these feeding activities is the associated act of spontaneous drinking which, as we have noted, disappears at once from a bird decerebrated after it is established and fails completely to develop in one decerebrated at hatching. Functionally this act ranks in immediate importance above pecking or even above seizing and swallowing, and is undoubtedly far more important than scratching in litter, yet it is perhaps the least deep-seated of all the feeding activities. Any attempt to account for the failure of representation of so fundamental an act in the underlying portions of the nervous system must be purely speculative. The suggestion may be permitted, however, that drinking is a necessary activity only in land forms. Feeding movements, on the other hand, occur as well in aquatic animals. If we assume that the establishment of function within the sub-cerebral nervous structures took place during the period when life was predominantly marine and when, therefore, deliberate drinking was unnecessary, we can see why the nervous mechanism of drinking might not have arisen within these lower nervous regions, but in a region, the cerebrum, of later development.

We do not feel disposed to enter upon any discussion of the influence of decerebration upon the complex activities listed by us under group *d*. We have described modifications of behavior which seem to us significant but offer them rather as contributions to knowledge than as the basis of definite conclusions.

The comparison of "shallow," "standard" and "deep" decerebration confirms the conception previously established for adult birds, that the impairment of function is more profound the more extensive the injury. There is still room for question, however, whether the marked effects of "deep" decerebration are due to interruption of important nerve pathways or whether they are secondary in character, resulting from disturbance of the temperature-regulating or some other "vital" mechanism.

The negligible impairment of function following unilateral decerebration, when not involving injury to the thalamus, demonstrates first, that the effects of our other procedures were not due to operative shock; and second, that one cerebral hemisphere suffices in birds for the usual range of their activities.

Our observations on a decerebellate chick are chiefly confirmatory of current views. Complete incoördination of the muscles concerned with locomotion, associated with marked and almost continuous struggling, was the most striking feature. There was a definite but limited control over head movements. Whether this was due to failure to ablate the entire cerebellum or whether there is such a limited control independently of the cerebellum can be decided only on the basis of further work.

SUMMARY

1. Three types of decerebration were performed on newly-hatched or very young chicks. These were "standard" decerebration, repetition of the usual operation as done on pigeons; "shallow" decerebration, removal of the pallium with avoidance, so far as possible, of injury to the corpora striata; "deep" decerebration, injury to the thalamus in addition to removal of the structures anterior. Unilateral decerebration of "standard" and of "deep" type was also performed.

2. The behavior of young normal chicks is considered in terms of four groups of activities: *a*, locomotor; *b*, self-cleaning; *c*, feeding; *d*, miscellaneous activities of a higher order. The age at which these appear is noted.

3. Chicks, decerebrated according to "standard" procedure immediately after hatching, develop locomotor and self-cleaning activities as early and substantially as efficiently as normals. They begin to peck about as early as normals but fail to progress beyond the act of pecking to successful seizing of food. Scratching in litter develops very slowly. Spontaneous drinking fails to appear. There is pronounced tendency to run toward moving objects. "Wildness" and fear are absent.

4. Chicks decerebrated between the third and eighth days, after the normal reactions are well established, revert to the condition of chicks decerebrated immediately after hatching. These chicks resume the activity of scratching in litter more promptly than it is developed in chicks that had not scratched at the time of decerebration. These chicks show a marked tendency for a few days to run in straight lines.

5. Chicks decerebrated after the eighth day show activities similar to those of other decerebrates except in the case of such as have to do with feeding. In those chicks there is markedly less pecking than in the other decerebrates and scratching fails completely to reappear.

6. Chicks in which the operation is confined to the ablation of the pallium, with a minimum of injury to the corpora striata, the so-called "shallow" decerebration, show only minor differences as compared with normals. There seems to be a more pronounced subservience to stimuli and less "wildness."

7. Chicks in which the operation includes injury to the thalamus, "deep" decerebration, appear weaker than the others and to have less secure equilibrium. The walking is unsteady and the act of preening is accomplished with difficulty. There is obvious impairment of

function when in a cool environment, suggesting a lowering of body temperature.

8. Unilateral decerebration has no demonstrable effect upon chicks except the loss of psychic vision on the side opposite the injury, with retention of the visual reflexes.

9. Ablation of the cerebellum brings about a condition of complete incoördination so far as locomotor movements are concerned, although there seems to be a limited power of coördination of the movements of the head. Compensatory movements are not readily demonstrable. There is much struggling and loud peeping.

10. The conclusion is drawn that the cerebrum has no necessary concern in the development and mediation of locomotor and self-cleaning activities in chicks. The successful accomplishment of feeding depends on the coöperation of the cerebrum, and the simpler phases of the act, pecking and scratching, are normally developed through the coöperation of the cerebrum, although if this is removed early enough in the life of the chick both may develop independently of it. A superior plasticity in early life is thus indicated.

11. The suggestion is offered that the complete disappearance of spontaneous drinking which follows decerebration may signify that this act, unnecessary in marine animals, may have developed comparatively late in evolutionary history, after the underlying parts of the nervous system were established in function, and concurrently with the development of the cerebrum.

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THE EFFECT ON BODY TEMPERATURE INDUCED BY THERMAL STIMULATION OF THE HEAT CENTER IN THE BRAIN OF THE CAT[1]

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Aronsohn and Sachs (1) in 1885 first performed the now well known heat puncture. They punctured the corpus striatum of the rabbit producing an increase of body temperature which they attributed to increased heat production from stimulation of the corpus striatum.

Hale White (2) in 1890 localized the temperature center in the corpus striatum and optic thalamus.

In 1912, Barbour (3) found that thermal stimulation of this center in the corpus striatum in rabbits produced changes in the rectal temperature. Heating the center lowered body temperature and cooling it raised the temperature.

The experiments of this paper were performed with the purpose of throwing light on two points:

1. To ascertain whether the mechanism discovered by Barbour (3) in the rabbit is peculiar to that animal; and
2. To determine the external landmarks of the heat center in the brain of the cat, an animal which is more suitable for certain experiments than the rabbit.

METHOD

The method used was essentially that used by Barbour (3) on rabbits. Aseptic operations were performed under ether anaesthesia. The brain was punctured with a double metal tube which carried a stream of water of the desired temperature. The tube was introduced and held in position by a metal cylinder screwed into the skull. The temperature of the water running into the tube was about 10°C. for cold and about 50°C. for hot. The body temperature was measured per rectum.

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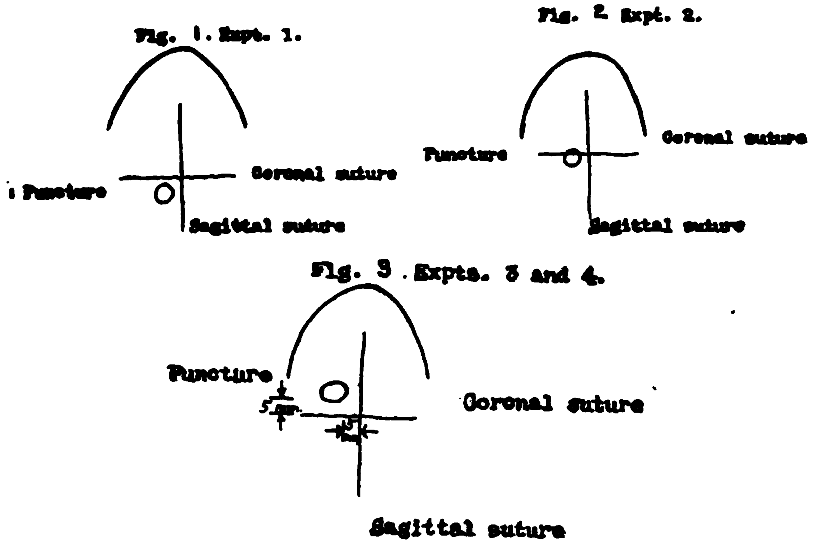
[1] The expense of this research was defrayed by the Loomis Medical Research Fund.

It should be emphasized that heating and cooling of the center was always carried out after complete recovery from anaesthesia. The failure of Sachs and Green (4) to demonstrate the heat center in cats may have been due to the use of an anaesthetic.

In each experiment time was allowed for the puncture fever to assert itself before applying thermal stimulation to the brain.

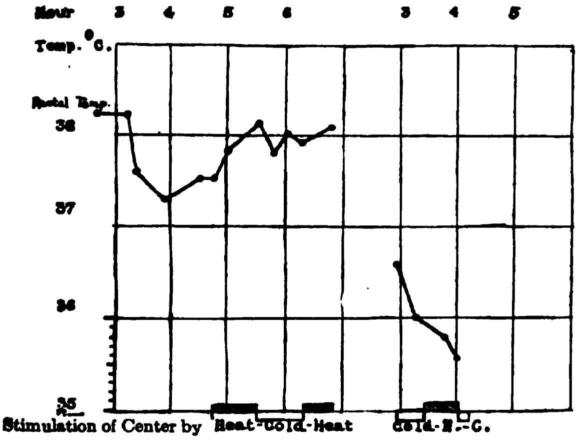
In experiments 1 and 2 no constant effects on the body temperature were obtained by heating and cooling the brain (see figs. 4 and 5). It was concluded therefore that the heat center had not been punctured and that the site of puncture used in these two experiments was not the correct one (figs. 1, 2).



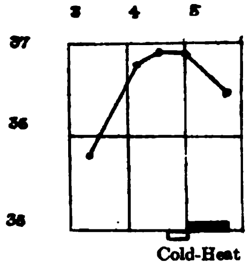
Diagrams showing site of brain puncture with reference to external landmarks. Figure 3 shows the correct external landmarks for puncture of the "heat center."

In experiments 3 and 4 constant results were obtained. Heating the brain caused a distinct fall in body temperature and cooling caused a distinct rise (see figs. 6 and 7). It was therefore concluded that in these two experiments the puncture reached the heat center. The punctures in these two cases were made 5 mm. lateral to the sagittal suture and an equal distance anterior to the coronal suture (fig. 3). A gross brain section made of the cat used in experiment 4 showed the puncture to penetrate the brain 1 mm. in front of the anterior border of the corpus striatum.

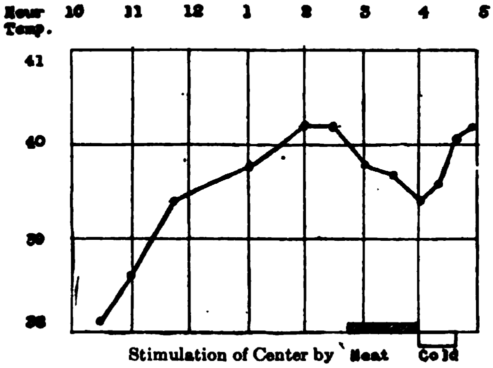
Expt. 1. Fig. 4
Site of Puncture shown in Fig. 1



Expt. 2. Fig. 5
Site of Puncture shown in Fig. 2.

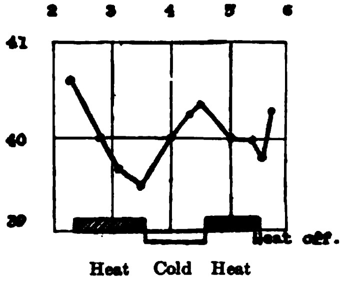


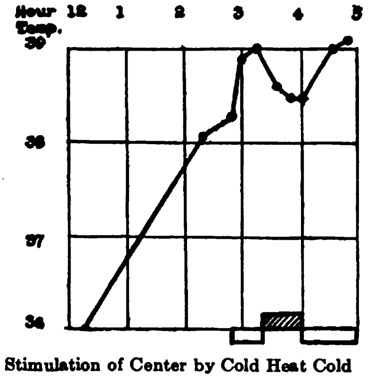
Expt. 3. Fig. 6
Site of Puncture shown in Fig. 3.



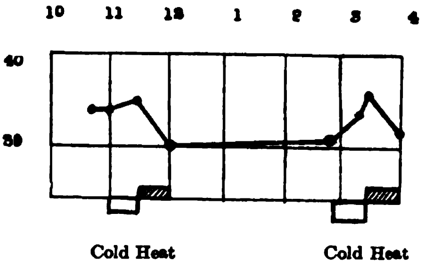
Puncture fever

Same animal on next day





Puncture fever.



Expt. 4. Fig. 7
Site of Puncture shown in Fig. 3

CONCLUSIONS

1. The "heat center" in the cat is located by the same external landmarks as in the rabbit, i.e., 5 mm. lateral to the sagittal suture and the same distance anterior to the coronal suture.

2. In the cat as in the rabbit, heating the heat center induces a lowering and cooling induces a rise in the body temperature.

3. Heating and cooling of the brain at other sites does not produce a similar constant effect.

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THE EFFECT ON THE VOLUME OF THE HIND LIMB INDUCED BY HEATING AND COOLING THE CORPUS STRIATUM OF THE RABBIT

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Barbour (1) in 1912 found that thermal stimulation of the heat center in the corpus striatum in rabbits produced changes in the rectal temperature. Heating the center lowered body temperature and cooling it raised the temperature. Barbour pointed out the usefulness of this mechanism by which it appears that fever would automatically lower body temperature, and vice versa, an abnormally low temperature would tend to be raised.

In 1914 Barbour and Prince (2) showed that these changes in temperature were due in part to changes in heat production, as they were able to demonstrate that thermal stimulation of the center produced changes in the carbon dioxide output, the oxygen consumption and the respiratory volume.

Barbour had previously (1) shown that the changes in temperature were associated also with changes in heat dissipation, for heating the center caused dilatation and cooling it caused constriction of the vessels of the ear.

The experiments of this paper were devised to ascertain:

1. Whether the "heat center" in the corpus striatum controls also a vasomotor mechanism in the hind limb.
2. Whether such a mechanism involves control mainly of the vessels in the skin.

METHOD

Rabbits were used. The corpus striatum was punctured according to the method described by Barbour (1) A double metal tube which carried a stream of water of the desired temperature was introduced and held in position by a metal cylinder screwed into the skull. The corpus striatum was reached by pushing the tube through an opening in the skull trephined at a point about 5 mm. from the sagittal suture and an equal distance in front of the coronal suture. The temperature of the water running into the tube was about 10°C. for cold and about 50°C. for hot.

In some experiments aseptic operations were performed under ether anaesthesia and the animals were allowed to come out from the ether to be used on the next day. When the animal was to be used only on the current day, paraldehyde was used (1.7 cc. per kilo body weight) and absolute asepsis was not considered necessary because the experiment did not last long enough for infection at the site of operation to become a factor influencing the results.

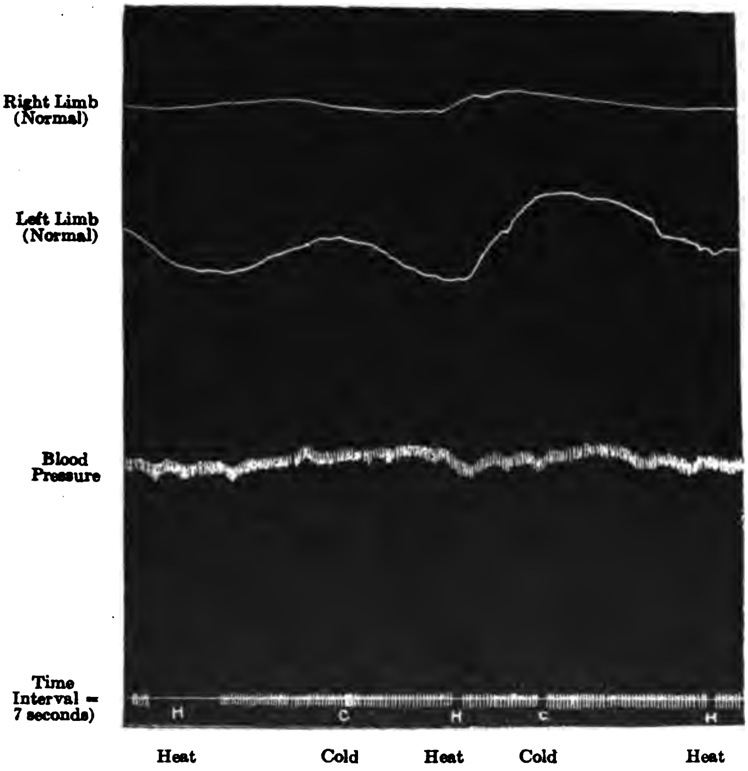

Fig. 1

In order that changes in the temperature of the environment should not disturb the experiment, the animals were kept under conditions

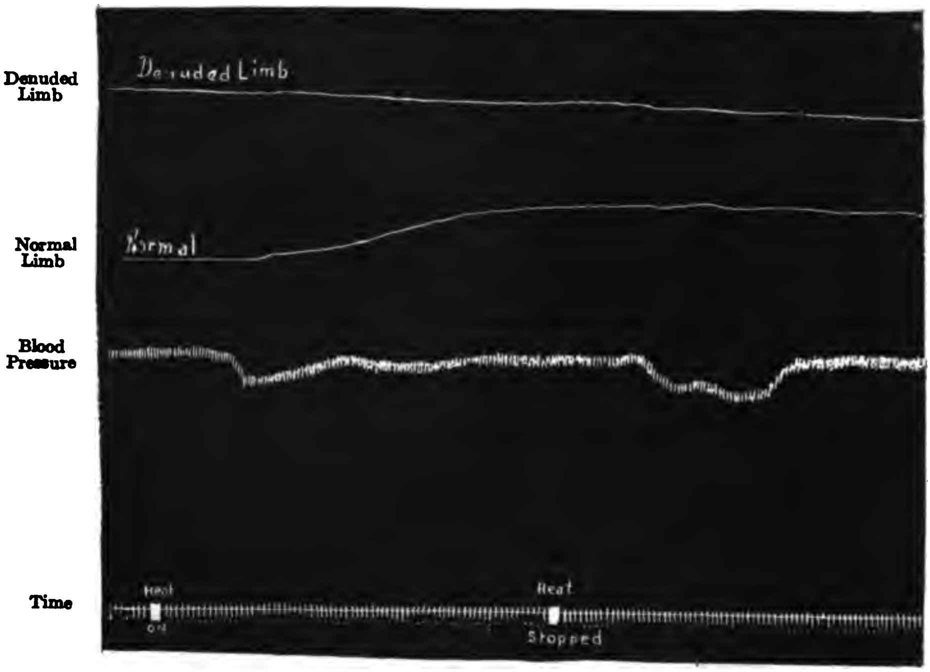
Denuded Limb
Denuded Limb
Normal Limb
Normal
Blood Pressure
Time
Heat on
Heat Stopped

Fig. 2

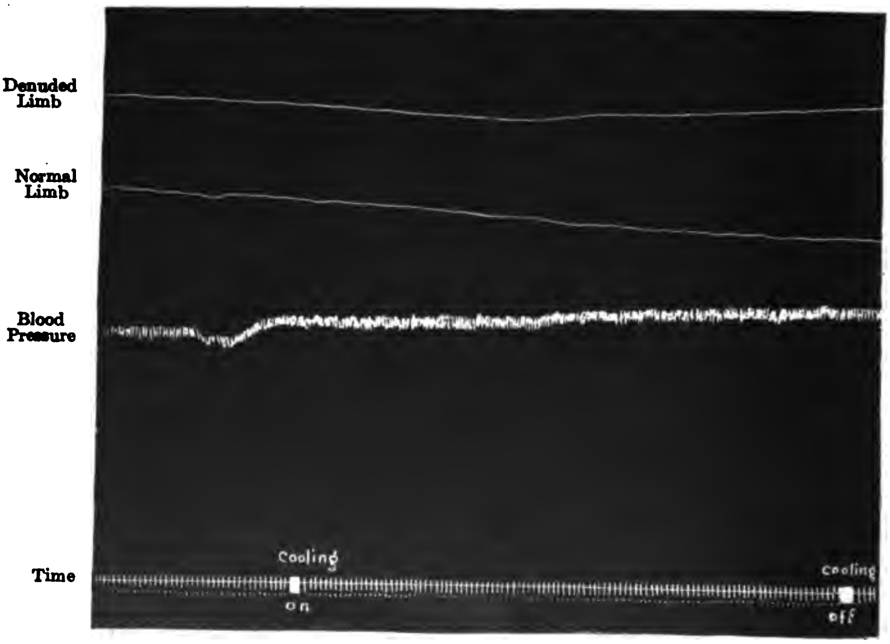
Denuded Limb
Normal Limb
Blood Pressure
Time
Cooling on
Cooling off

Fig. 3

conducive to a constant body temperature by being placed above a warm pan, but separated from it by a wooden stage. Blood pressure records were kept during all the experiments as a control of the volume changes in the limb.

In order to determine whether the changes in the volume of the limb were mainly effected by the skin vessels, in some experiments the skin was carefully removed from one leg while the other leg was left intact, and synchronous records were obtained from both legs. In removing the skin, special care was taken not to injure the subcutaneous veins.

Changes in the volume of the hind limb were measured by a plethysmograph connected to a lever registering on a smoked drum. Vasoconstriction in the limb is indicated by a drop in the curve and vasodilation is indicated by a rise.

The graphic records (fig. 1) show distinctly that heating the center produces dilatation of the vessels of the hind limb and cooling the center causes vasoconstriction. Figures 2 and 3 show that these changes do not take place in the denuded limb.

CONCLUSIONS

1. Cooling the "heat center" in the corpus striatum in rabbits causes vasoconstriction in the hind limb.
2. Heating the center causes vasodilatation in the hind limb.
3. The skin vessels are the vessels mainly concerned in these effects, as shown by negative results with the limb denuded.
4. Further evidence is furnished of a central mechanism in the corpus striatum which exercises control over the vasomotor centers.

The expenses of the research reported in this paper were defrayed by the Loomis Medical Research Fund.

The authors desire to express gratitude to Dr. Yandell Henderson and Dr. H. G. Barbour for their advice and assistance.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLVIII. Studies in Water Drinking

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In this work a study has been made (1) of the influence of copious water drinking with the meals upon gastric secretion and the emptying time of the stomach; (2) of the emptying time of water from the empty stomach; (3) of gastric stimulation by water; (4) of the latent period of the human gastric glands when stimulated by water; and (5) of gastric glandular fatigue.

WATER DRINKING WITH THE MEALS AND GASTRIC SECRETION

That water is a gastric stimulant has been pointed out by many investigators, e.g., Heidenhain (1), Sanotski (2), Pavlov (3), Krshyschkowshy (4), Foster and Lambert (5), Bergeim, Refuss and Hawk (6), Sawtch and Zeliony (7), Carlson, Orr and Brinkman (8). It has been shown by Foster and Lambert that water not only stimulates gastric secretion when given alone but that it also stimulates the gastric glands when it is given with food. Although, as Pavlov has shown, the stimulation is chemical, it has been demonstrated by Carlson, Orr and Brinkman that the psychic or thirst factor is of importance in the stimulation of the gastric glands by water. With this point in view the work of Foster and Lambert has been repeated.

Methods. Dogs with Pavlov accessory stomachs were prepared and put on a diet of 300 grams of meat and 800 cc. of water per day. The dogs were given two meals each day, one at 8.00 a.m., the other at 3.00 p.m. The juice was collected after each meal over a period of three hours. When water was given with the meal, the amount was 300 cc. An attempt to give the dogs, which were rather small, 500 cc. of water with the meal caused evident discomfort. So 300 cc. of water was the amount decided upon to be used in this series of experiments. In order to eliminate a possible factor of normal variation, the water meals were varied; some days water would be given with both meals' then with neither or only with the morning or afternoon meal. The water when not given with the meal was given after the evening meal and at 11.00 p.m. The dogs were always offered water before the meal to see if thirst was present. It was refused in every instance. The water was always given by tube as the dogs would not drink any of it. The dogs, after training, took the tube without any apparent discomfort and would even allow the tube to remain in position indefinitely.

In table 1 the degree of acidity is expressed in clinical units (the number of cubic centimeters of N/10 NaOH required to neutralize 100 cc. of gastric contents). The peptic activity is expressed in millimeters of digested coagulated egg white, according to Schiff's modification of Mett's method. The length of the period of diet and experimentation was twelve days, so that the figures in the table represent the average of three days' results under each procedure.

The results upon these five dogs (table 1) show that there was always an increase in the amount of juice, but the increase in acidity did not occur in every instance. The amount of pepsin generally remained the same but often when the amount of juice was markedly increased, the peptic activity would be reduced. Although on a diet, these dogs showed some variation in the amount and acidity of the gastric juice from day to day, the peptic activity was more constant. By varying the procedure as described above, this factor of daily variation was eliminated.

It is apparent from these results that when factors of thirst and normal variation are controlled there is an increase in the total amount of gastric juice and in the free and total acidity of this gastric juice upon the ingestion of water with the meals, confirming the results of Foster and Lambert (5). It is interesting to note from the table that when the water was given with the afternoon meal, in every case but dog I, the amount of juice was increased over the amount when the water was given with the morning meal. The dogs were probably slightly thirsty, not having had any water between meals, although they refused water offered to them before the meal. It, however, must be recalled that the water was given by tube which is said to eliminate the psychic factor. Two explanations are possible: first, that the increased stimulation by water during thirst is not due to a psychic secretion but to an increased irritability of the gland cells to respond to the chemical stimulation of the water; or second, the psychic influence is not eliminated by the stomach tube because the sensation of cooling the throat, oesophagus and stomach, which is present with the tube in, is still experienced. The factor of dilution of the blood as suggested by Carlson, Orr and Brinkman (8) may also be a factor concerned in this increase.

TABLE 1

Influence of water drinking with the meals on gastric secretion

		PROCEDURE	GASTRIC JUICE							
			No water with meal				H_2O with meal			
			Amount of juice 3 hours	Acidity		Pepsin	Amount of juice 3 hours	Acidity		Pepsin
				Free	Total			Free	Total	
			cc.			*mm.*	*cc.*			*mm.*
Dog I..	1	H_2O with morning meal	16	57	82	1.8	22	60	85	1.8
	2	H_2O with afternoon meal	13	37	72	2.0	19	65	97	1.8
	3	H_2O with both meals					24	60	87	2.0
	4	No H_2O with either meal	13	62	82	1.8				
Dog II.	1	H_2O with morning meal	20	72	107	1.5	34	97	110	1.25
	2	H_2O with afternoon meal	20	52	77	1.5	50	112	125	1.25
	3	H_2O with both meals					35	112	122	1.00
	4	No H_2O with either meal	21	75	102	1.5				
Dog III.	1	H_2O with morning meal	58	127	135	2.0	72	125	132	1.7
	2	H_2O with afternoon meal	60	117	125	2.0	75	125	130	1.7
	3	H_2O with both meals					71	120	130	2.0
	4	No H_2O with either meal	56	117	125	2.0				
Dog IV.	1	H_2O with morning meal	17	100	107	1.0	25	107	120	1.0
	2	H_2O with afternoon meal	18	97	105	1.0	28	105	122	1.0
	3	H_2O with both meals					24	105	120	1.0
	4	No H_2O with either meal	17	100	110	1.0				
Dog V..	1	H_2O with morning meal	24	95	105	2.0	35	100	115	2.0
	2	H_2O with afternoon meal	25	92	105	2.25	39	97	110	2.0
	3	H_2O with both meals					34	100	112	2.0
	4	No H_2O with either meal	23	95	105	2.0				

Water drinking with the meals and gastric stimulation: man. A series of experiments was next conducted to see if stimulation resulted in man upon drinking copious amounts of water with the meal. The man experimented upon was placed upon a diet of two meals a day, one at 11.00 a.m., the other at 5.30 p.m. The meal consisted of 125 grams of graham crackers, 50 grams peanut butter, 300 cc. milk and 400 cc. of water. This was the amount of fluid normally taken during the meal by this person. The diet lasted eight days. Eight hundred cubic centimeters of water were ingested when the amount was called "copious." The "copious" water meal was varied so that two of the days the meals were of "moderate" water (400 cc.), or of "copious" water (800 cc.), or of "copious" water with the first meal or with the second meal. This was done for the purpose of eliminating the chance of any normal variation in gastric secretion. The factor of thirst was controlled by keeping the daily amount of water intake constant and by drinking certain amounts at 7.00 a.m. and at 11.00 p.m. Samples of the gastric contents were withdrawn and analyzed for acidity. The emptying time of the stomach was recorded.

TABLE 2

Subject I

TIME	400 cc. H_2O with meal		800 cc. H_2O with meal		REMARKS
	Acidity		Acidity		
	Free	Total	Free	Total	
Residuum 7.00 a.m.	17	22	30	40	Meal
8.00 a.m.	0	17	0	23	
8.30 a.m.	1	25	7	40	
9.00 a.m.	1	32	15	80	
9.30 a.m.	17	47	30	100	
10.00 a.m.	25	82	42	115	
10.15 a.m.			67	122	
10.30 a.m.	40	105	70	112	
10.45 a.m.	45	115	52	85	
11.00 a.m.	45	115	60	82	
11.15 a.m.	57	100	60	82	With 800 cc. stomach empty
11.30 a.m.	62	95	60	75	
11.45 a.m.	62	95	45	50	
12.00 noon	42	55	35	42	With 400 cc. stomach empty
12.15 p.m.	35	40			

The following table 2 shows typical results obtained where 400 cc. and 800 cc. of water were taken with the meal.

Table 2 shows that there is only a slight increase in the free and total acidity of the gastric contents and that the rise in the acidity comes sooner and the stomach empties quicker when "copious" water is ingested with the meal. This occurred in every case in this one individual.

This experiment was repeated on (Mr. F. V.) a gastric fistula case. The meal consisted of bread,[1] potatoes and coarsely ground meat mixed with milk to the consistency of a "mush," 300 cc. of this mixture being injected into the stomach via the fistula. The amounts of water injected after the injection of this food mixture were 150 cc. and 450 cc. The following table shows four typical results obtained.

The interchanging of the 150 cc. and 450 cc. water meals eliminates the possibility of a misinterpretation of the results. Mr. F. V. reports from personal observation that when he puts water into his stomach "the food leaves quicker." These results, shown in table 3, not only verify the subject's observation, but practically duplicate the results upon subject I (table 2). There is a definite increase in the acidity and a noticeable decrease in the emptying time of the stomach when the larger amount of water was taken with the meal.

TABLE 3

Influence of water drinking with the meal on acidity and emptying time

TEST	150 cc. H_2O WITH MEAL			450 cc. H_2O WITH MEAL			REMARKS
	Acidity		Emptying	Acidity		Emptying	
	Free	Total	Time	Free	Total	Time	
I	*87	112	1 hr. 25 min.	92	122	1 hr. 10 min.	
III	102	120	1 hr. 30 min.	110	127	1 hr. 15 min.	
IV	95	110	1 hr. 30 min.	97	125	1 hr. 10 min.	
VI	95	117	1 hr. 45 min.	100	122	1 hr. 30 min.	
VII	90	112	2 hrs.	112	127	1 hr. 40 min.	700 cc. used instead of 450 cc.
IX			1 hr. 50 min.			1 hr. 35 min.	700 cc. used instead of 450 cc.

* Acidities are maximum.

Not being able to get other men to work upon, it was decided to repeat this work upon dogs and cats. The dogs were studied by means of gastric and duodenal fistulas and the cats by means of X-ray. The cats were fed 50 grams of salmon mixed with 10 grams of barium sulphate. The dogs were fed 150 grams of finely ground cooked meat mixed with 50 cc. of water.

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[1] On two days the meals consisted chiefly of meat, Mr. F. V. substituting this without my knowledge. The emptying time of each meal with large and small amounts of water was two hours. In other tests the above meal was strictly adhered to.

The first gushes of gastric contents appeared in from 15 to 45 minutes after the ingestion. Chyme appeared sooner in dog I and later in dog IV. The time for its appearance was fairly regular in each of the four dogs; in dog I, 15 to 20 minutes; in dog II, 20 to 30 minutes; in dog III, 30 to 40 minutes; and in dog IV, 35 to 45 minutes. Moritz (9) and Cannon (10) observed that the exit began about three-quarters of an hour after feeding, while Cohnheim and Lang (12) report that the exit begins in 15 minutes. Cannon explains the discrepancy to a difference in the consistency of the food. But here food of the same consistency was fed to four dogs and each showed a different exit time. Might not the discrepancy in the results of Cohnheim-Lang and Moritz-Cannon be explained by an individual variability in the exit time of the stomach in the dogs they worked upon?

TABLE 4

Influence of water drinking with the meal on acidity and emptying time

DOG	50 cc. H_2O WITH MEAL			400 cc. H_2O WITH MEAL			REMARKS
	Acidity		Emptying	Acidity		Emptying	
	Free	Total	Time	Free	Total	Time	
I	100	132	3 hr.	105	137	2½ hr.	
II	77	122	3½ hr.	85	125	3 hr.	This dog two out of six trials showed no decreased emptying
III*	80	120	3½ hr.	80	120	3¼ hr.	
IV	75	117	4 hr.	82	122	3½ hr.	

* This dog in no instance showed a decreased emptying time with 400 cc. H_2O. The dog may not have responded to water stimulation. It died before this could be verified.

The results obtained upon the cats were variable and unsatisfactory and do not warrant consideration.

These observations upon dogs and man suggest that the emptying time of the stomach is decreased by the ingestion of "copious" amounts of water with the meals. This confirms Cannon's (10) and Hedblom's observation that "the dilution of the protein food tends toward a more rapid discharge of the protein from the stomach." Attention must be called to the fact that in the experiment upon the dogs the water and meat was mixed before feeding while in man the water was taken at intervals during the meal, which would have a tendency to mix the food and water and to retard the emptying of water from the stomach which, according to Cohnheim (12), does not take place during digestion. On this point, however, the literature does not agree as Leven and Barret (13) have found that, although water empties rapidly from the resting stomach, the discharge of water from the stomach when taken with food is retarded. Groebbels (14) states that bread followed by water shortens the time of the digestion of the bread. Gabrilowitch (14) states that when water is mixed with meat the water passes out and the meat follows the customary digestion.

The following table shows the results obtained for the discharge of 400 cc. H_2O from the empty and full stomach.

TABLE 5

Emptying time of water when taken into empty and full stomach

DOG	EMPTYING TIME OF 400 cc. H_2O FROM THE EMPTY STOMACH		EMPTYING TIME OF 400 cc, WITH 150 GRAMS OF MEAT IN STOMACH	
	Maximum	Minimum	Maximum	Minimum
I	45 min.	30 min.	1 hr.	45 min.
II	55 min.	40 min.	1 hr. 20 min.	1 hr.
III	1 hr.	50 min.	1 hr. 15 min.	1 hr.
IV	1 hr. 15 min.	55 min.	1 hr. 45 min.	1 hr. 25 min.

Time was taken when 400 cc. of fluid were obtained from the fistula. This, of course, contained particles of food and some gastric juice.

Three tests were made in each dog. The 400 cc. of water were given directly after the meat. Without delay water came from the duodenal fistula, but did not "pour" through the fistula as described by Cohnheim (12). It did, however, come in copious gushes of from 10 to 20 cc. which gradually decreased in quantity.

The above experiment was repeated upon Mr. F. V., a gastric fistula case. The normal emptying time of 450 cc. of H_2O from the empty stomach of this man was 15 to 20 minutes. When the same amount of water (450 cc.) was put into the stomach containing food the emptying time of the water from the stomach was 40 minutes,[2] as shown by the average of five tests. In other words, with food in the stomach the emptying of 450 cc. of water was retarded 25 minutes.

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[2] It was impossible to make as accurate observations here as were made in the dogs. A judgment had to be made from the ease with which the sample was obtained from the stomach and from the absence of food in this sample. In collecting the samples Mr. F. V. places a small stiff-rubber tube through the larger rubber tube, which is always kept in place; he then leans forward and the contents run out through the stiff rubber tube.

The observations upon these dogs and upon Mr. F. V. support Léven and Barret's findings. There is always retardation, but the degree varies in different dogs and to a less extent in the same dog. This retardation in the emptying time of the water is unquestionably caused by the water mixing with the food, rendering the food more dilute. This being the case, then, the decreased emptying time of the stomach with "copious" water with the meal is due to the dilution of the stomach contents, facilitating digestion and evacuation.

EMPTYING TIME OF WATER FROM THE EMPTY STOMACH

The emptying time of water in those four dogs varied from 30 minutes to 1 hour. It varied slightly from time to time in the same dog. The water began to enter the intestine practically as soon as it reached the stomach, i.e., 5 to 10 seconds after it was introduced per os or per gastric fistula. In every dog it passed from the stomach in single gushes at varying intervals of from 10 to 30 seconds, each gush delivering from 5 to 30 cc. of water. These gushes as to time seemed to occur in groups, e.g., several of 10 second intervals would occur, then several of 15 seconds, then several of 12 seconds, and between these there might be interposed one or two of 5 seconds, or of 30 second intervals. There was evidence of rhythm. The gushes became slower as the stomach became empty. The water during the first few minutes was practically neutral, but after five minutes the acidity ranged from .02 per cent to .04 per cent and increased steadily.

These observations confirm those of Moritz (9) and London and Sulima (11) except as to the time between gushes. They report that the interval between gushes is too short to be accounted for by peristaltic movements. But from the observations made in this series of experiments the gushes could easily correspond to the occurrence of the peristaltic waves or stomach contractions, as reported by Von Mering (21) in 1893.[3]

In man, as will be seen from table 6, the emptying time of water rom the empty stomach also varies. In normal individuals, enjoying perfect health, the emptying time varied from 400 cc. in 15 minutes to 0 cc. in 15 minutes. The cases of gastric fistula, gastric ulcer and gastro-enterostomy emptied 360 cc. in 15 minutes. The rate of emptying generally does not vary more than 40 cc. per 15 minutes period in the same individual when 400 cc. are drunk. There was one exception to this, however, occurring in subject W, table 6.

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[3] I believe that the operative technique is of importance in explaining the conflicting observations. See a former paper for a description of the methods used in making the duodenal fistula. (This Journal, 1918, xlvi, 340.)

TABLE 6

Gastric stimulation by water in twenty men. Drinking 400 cc. H_2O

SUBJECTS	AVERAGE AMOUNT OF FLUID OBTAINED BY STOMACH TUBE AFTER 15 MINUTES	ACIDITY OF CONTINUOUS GASTRIC SECRETION	AVERAGE MAXIMUM ACIDITY AFTER INGESTION OF 400 cc. H_2O	REMARKS
	cc.			
I	300	25.0	60.0	Similar response to Ewald test meal
K.N.	280	30.0	70.0	Similar response to Ewald test meal
H.	270	55.0	95.0	Similar response to Ewald test meal
R.	340	45.0	115.0	
M.	200	47.5	62.5	
S.	200–230	7.0–22.5	52.5	Similar response to Ewald test meal
K.	355	20.0	55.0	Similar response to Ewald test meal
Se.*	400*	35.0	55.0–65.0	Similar response to Ewald test meal
Ma.	300	37.5	57.0	
N.	150	10.0	22.5	Responded to Ewald meal. Total acidity 40
W.	110–220	10.0–17.5	17.5–47.5	Quite variable: Six tests were made upon this person
C.	100	10.0	20.0	Responded to Ewald meal. Total acidity 50
L.K.	170	37.5	45.0	Responded to Ewald meal. Total acidity 50 to 60
O.	150	35.0–50.0	40.0–55.0	Responded to Ewald meal. Total acidity 50 to 60
G.	140	35.0	37.5	
Gr.	130	27.5	32.5	Responded to Ewald meal. Total acidity 50 to 55
F.	35	80.0	95	Gastric fistula
Mi.	30	80.0 (Contents)	105	Gastric ulcer
T.	300–400	40	30	Free acidity 10—Gastric carcinoma
J.	40	45	70	Gastro-enterostomy, 7 years standing

* This subject would withdraw from his stomach after drinking 400 cc. of water (at the end of 15 minutes) 415 to 420 cc. of contents. In other words withdrew from his stomach after 15 minutes more fluid than he put into it.

Holyknecht (16) states that 200 cc. of water are evacuated in 60 minutes, and Kastle (17) gives 70 minutes as the emptying time for the same amount. Ernst (18), Waldeyer (19) and Kauffman (20) have produced evidence of a "Rinne" or trough in the lesser curvature, which was also pointed out by Cohnheim (12), in case the stomach contains food. Scheunert (22) from experiments on the horse's stomach states that liquid in the distended stomach penetrates along the gastric wall. Von Mering (21) reports that practically 500 cc. of water are emptied from the stomach in 15 minutes. Bergeim, Refuss and Hawk (6) cite one case in man in which 500 cc. of water left the stomach in from 10 to 20 minutes. Such marked discrepancies in the literature are apparently explained by the wide range in individual variability pointed out by the series of results in this study on man and dog.

That water leaves the stomach much sooner than milk or food cannot be questioned; but it is not emptied as fast as Cohnheim's and Von Mering's observations would lead one to believe. As judged from this series the emptying of water from the normal human stomach varies, conservatively, from 400 cc. to 100 cc. in 15 minutes.

GASTRIC STIMULATION BY WATER. MAN

It has been shown by Bergeim, Refuss and Hawk (6) that the human stomach is stimulated by water and further they report that "in the average normal individual water produces fully as great a stimulation as does the Ewald test meal." While studying the gastric secretion of several normal men, it was observed that some of these failed to respond, or responded but poorly, to gastric stimulation by water and that these stomachs emptied water fast as compared to those that gave a marked response to stimulation by water. This observation has been extended and study has been made of the gastric secretion of twenty men, seventeen of them reporting normal gastric histories.

Methods. All of the tests were made in the morning before any food had been ingested and from two to three hours after the last drink of water. The stomach was emptied, the continuous secretion taken for two or three 15 minute periods, 400 cc. of cool tap water were drunk, the stomach completely emptied after 15 minutes and every 15 minutes thereafter for 2 hours. The stomach was not emptied at the end of 15 minutes if the results were to be compared with an Ewald meal, in which case samples were withdrawn for analysis. At least three tests were made upon each individual.

Figures 1 to 7 inclusive show the type of stimulation resulting in some of my subjects. In table 6 is recorded a summary of abstracted results of the emptying time of water from the empty stomach and the resulting stimulation in each of the twenty subjects studied.

The liquid taken from the stomach 15 minutes after drinking was generally bile-tinged (Gmelin's test), indicating either that some of it came from the intestine or that bile was regurgitated as the stomach was emptied for the bile appeared in the last portion withdrawn. The amount of liquid drawn from the stomach never varied more than 40 cc. in the same individual. It was observed that on warm days the water left the stomach quicker. This factor was controlled as well as possible. The mechanism of this observation was not studied. That the stomach was empty was always verified by blowing into the tube; if bubbles were felt, the stomach was not empty; if a swelling sensation was experienced, the stomach was empty. During the time of aspiration the body was rotated so that the tip of the tube would lie in the most dependent portion of the stomach cavity

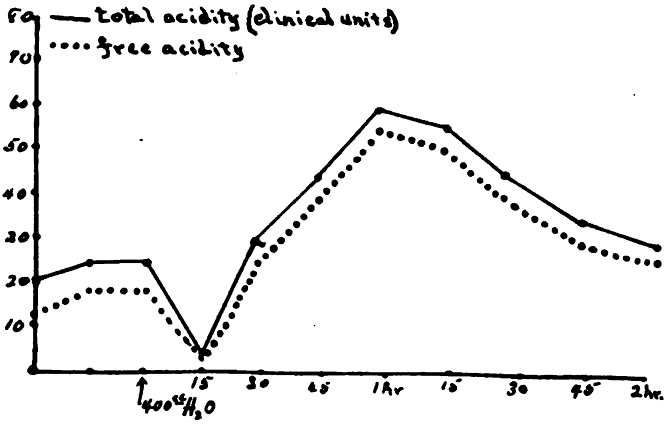

Fig. 1. Many tests were performed upon this subject. The response to water varied but little and was practically the same as was obtained with an Ewald test meal. On an average 300 cc. of water were taken from his stomach at the end of 15 minutes; 400 cc. were emptied in 1 hour.

The results upon the normal individuals indicate that the stimulatory power of water in the human stomach varies noticeably with different individuals, some responding markedly and others practically not at all. It is also apparent that those stomachs which empty the

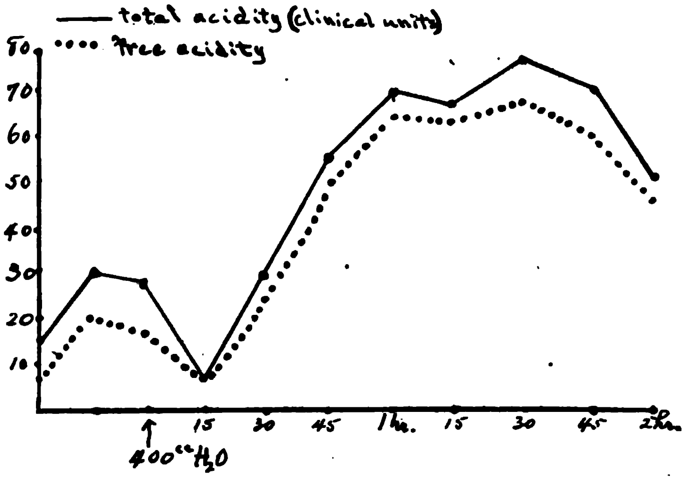

Fig. 2. This subject, K. N., always showed marked stimulation to water On the average 280 cc. were taken from his stomach at the end of 15 minutes

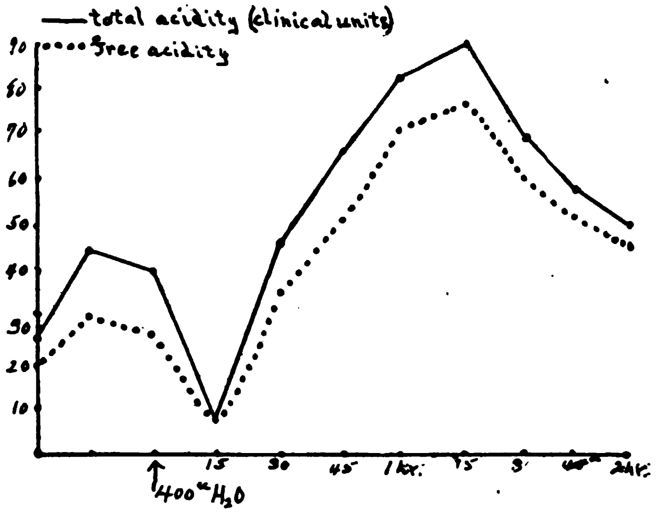

Fig. 3. The response here is also marked; 350 cc. were taken from his stomach at the end of 15 minutes.

water quickly do not respond to stimulation by water. The amount of water regained from the stomach after 15 minutes seems from the data in this study to be diagnostic of whether stimulation will occur or not. Bergeim, Refuss and Hawk (6), however, report a marked response in one person who emptied 500 cc. in 10 to 20 minutes which would be quite an exception to my results, except in the pathological cases I present. These investigators, on the other hand, did not measure the continuous secretion previous to the ingestion of the water, which questions the validity of such a stimulation.

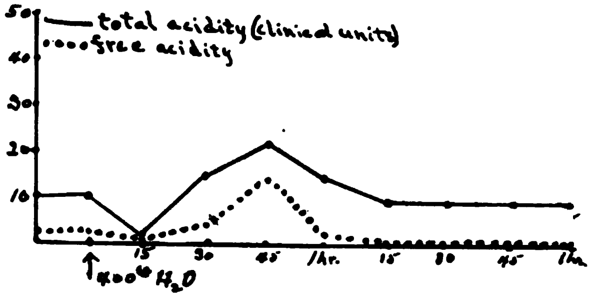

Fig. 4. This subject N shows practically no response to water, yet he gives a total acidity of 40 with the Ewald test meal at the end of an hour. Only 100 cc. of water were obtained from his stomach at the end of 15 minutes.

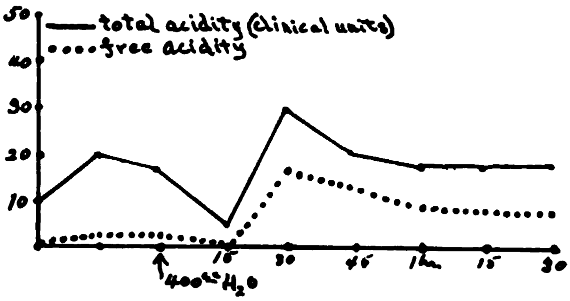

Fig. 5. This subject C shows practically no response to water; 100 cc. were obtained from his stomach at the end of 15 minutes. He gave a total acidity of 50 to an Ewald meal at the end of 1 hour.

LATENT PERIOD OF THE HUMAN GASTRIC GLANDS WHEN STIMULATED BY WATER

Pavlov has shown that in dogs the latent period of the gastric glands when stimulated by water is 5 minutes. This has been confirmed in this laboratory by Dr. G. F. Sutherland and myself, working separately. The latent period may be as long as 15 minutes. Bergeim, Refuss and Hawk (6) working upon one man state that "it was impossible to demonstrate any latent period for the human gastric glands." As some observations in the series of men suggested that there was a latent period and as Bergeim and his colleagues in their experiment did not take into consideration the continuous secretion of the stomach and used water for a lavage just before using it for a stimulus, it was decided to investigate the correctness of their conclusion.

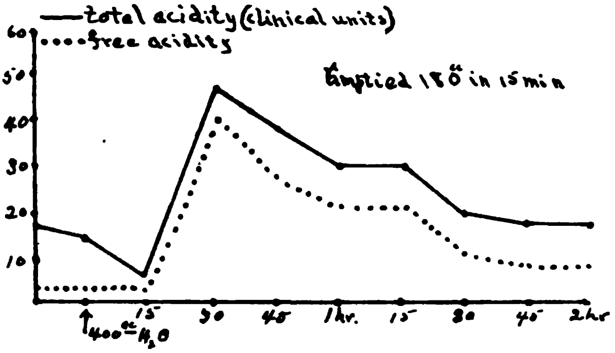

Fig. 6

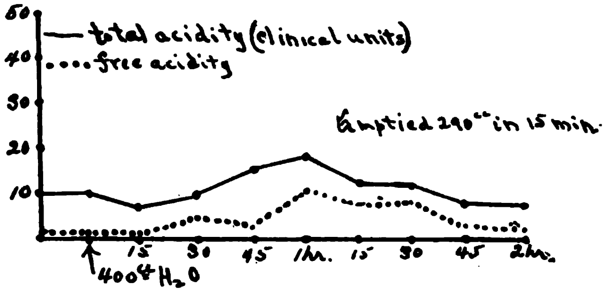

Fig. 7

Figs. 6 and 7. In subject W there was quite a noticeable variation in the emptying time. Figure 6 shows the gastric response when 220 cc. were taken from his stomach at the end of 15 minutes and figure 7 shows the response when 110 cc. were taken from his stomach at the end of 15 minutes.

It may be seen from the graphs in part 3 that the residuum is generally lower in acidity than the continuous secretion, due as suggested by Doctor Carlson to dilution by saliva and neutralization by ammonia (24). So the acidity of the residuum cannot be taken as the acidity of the continuous secretion. In this series of experiments, then, the stomach was emptied, the continuous secretion taken for three periods, 400 cc. of water were drunk and pumped out simultaneously. This never took more than 1½ minute. The stomach was then emptied completely every minute for 10 minutes and then every 5 to 10 minutes for 1½ hour. These experiments were done upon three men, in two by means of the stomach tube, the third being a gastric fistula case (Mr. F. V.) (23). Three tests were made upon the same individual. Figures 8, 9, 10 show typical results upon the three men used.

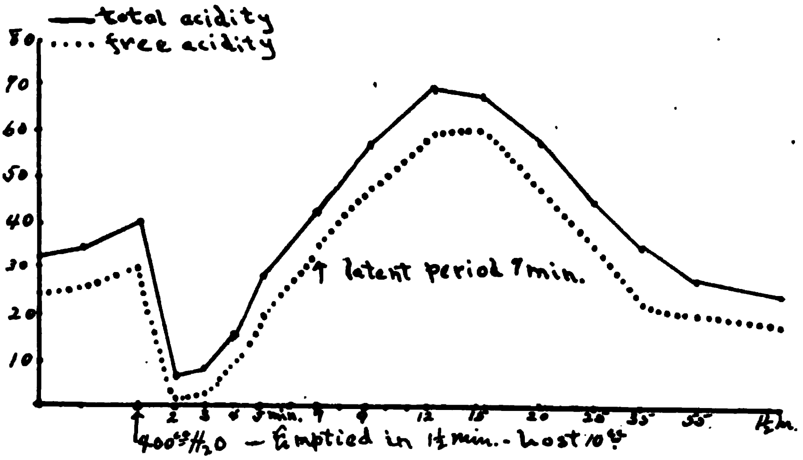

Fig. 8

These graphs (figs. 8, 9, 10) show that there is a latent period of from 5 to 10 minutes when the human gastric glands are stimulated by water, which corresponds to Pavlov's findings in the dog. In subject I the latent period for meat broth was 5 minutes. In subject F. V. the latent period of the psychic secretion is from 4 to 7 minutes. (See Carlson: Control of hunger in health and disease, Chicago, 1916). It is apparent from figures 9 and 10 how absence of a latent period could be mistaken, if the acidity of the residuum was taken as the acidity of the continuous secretion.

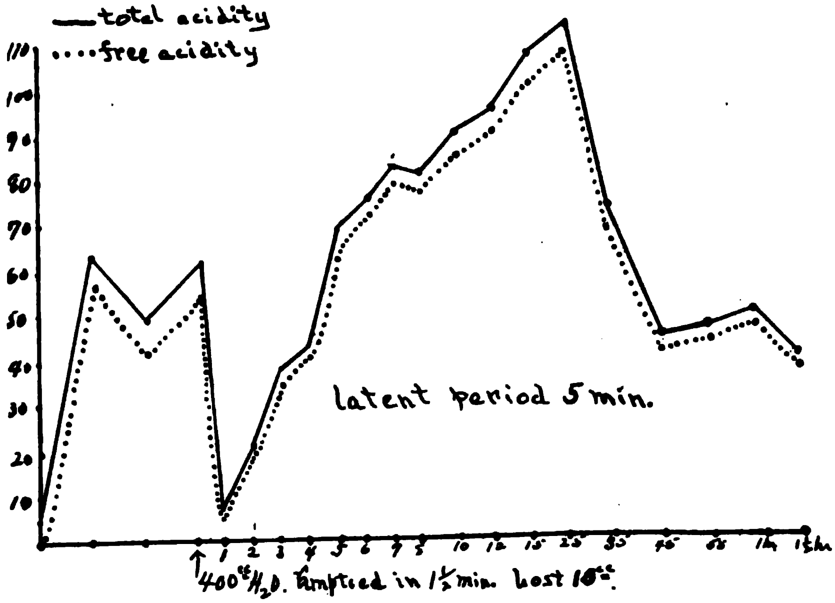
—— total acidity
···· free acidity
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
10
12
15
25
35
45
55
1h
1¼h
latent period 5 min.
400cc H2O. Emptied in 1½ min. Lost 10cc.

Fig. 9

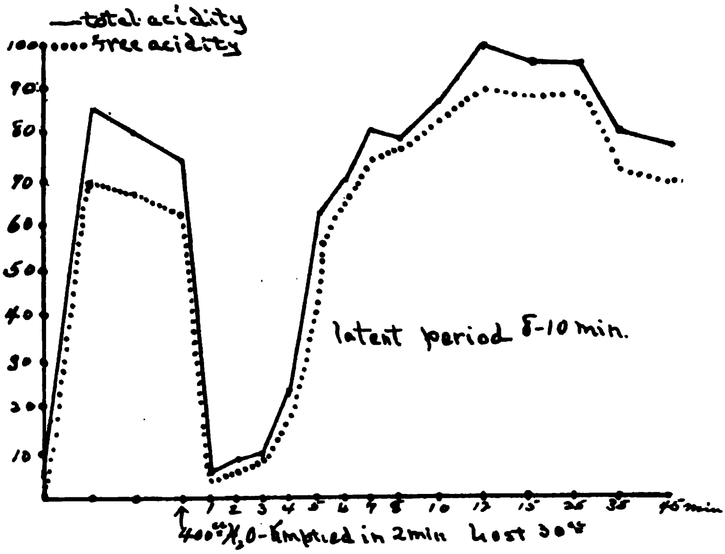
—— total acidity
···· free acidity
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
10
12
15
25
35
45 min
latent period 8-10 min.
400cc H2O-Emptied in 2 min Lost 30cc

Fig. 10

GASTRIC GLANDULAR FATIGUE

Foster and Lambert (5) from the results of some of their experiments suggest the presence of gastric gland fatigue. Bergeim, Refuss and Hawk (6), using water as a stimulant, state that "it is impossible to demonstrate any pronounced glandular fatigue in the human stomach." In this study an attempt has been made to fatigue the gastric glands of man and dog by stimulating with water and by the use of gastrin. Observations have also been made upon the possibility of fatiguing the psychic secretion.

TABLE 7

Gastric stimulation by water at two hour intervals

PROCEDURE 700 cc. H_2O	DOG I			DOG II			REMARKS
	Gastric juice			Gastric juice			
	Amount	Free acidity	Total acidity	Amount	Free acidity	Total acidity	
	cc.			*cc.*			
8.00–10.00	4.0	42	70	3	75	82	Dog had been kept from food and water for 24 hours previous to experiment
12.00	12.0	70	85	9	90	110	
2.00	10.0	75	87	12	95	112	
4.00	13	67	80	11	82	107	
6.00	11	70	85	13	87	112	
8.00	11.5	70	82	12	92	115	
10.00	12.5	72	80	10	90	110	

* Continuous secretion taken from 8.00 to 10.00 a.m.

In the first series of experiments 700 cc. of water were drunk at intervals of 2½ hours for a period of 10 hours. The stomach was completely emptied at the end of 15 minutes. The gastric juice was collected every 15 minutes and titrated for free and total acidity. Figure 11 shows the result of this procedure.

No evidence of gastric gland fatigue is manifested in the results obtained, of which figure 11 is a typical example.

Table 7 shows the results upon two dogs, 400 cc. of water being given at 2 hour intervals over a period of 10 hours.

There is no evidence of glandular fatigue when the stimulation is produced by water.

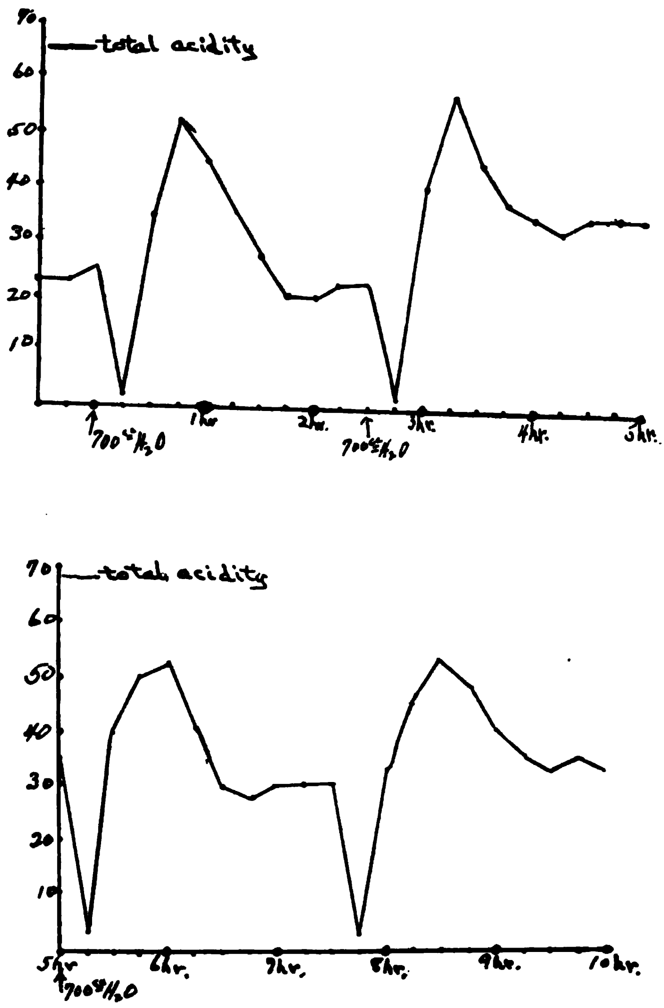

Fig. 11

It was next decided to attempt to produce fatigue of the gastric glands by injecting gastrin[4] every 2 hours over a long period of time. One cubic centimeter of gastrin was injected every two hours with the results shown in tables 8 and 9.

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[4] Dr. F. C. Koch kindly furnished me with the gastrin used in these experiments.

TABLE 8

Gastric stimulation by gastrin infected at intervals of two hours

1 cc. GASTRIN PROCEDURE	GASTRIC JUICE: DOG I				REMARKS
	Amount	Free acidity	Total acidity	Pepsin	
	cc.			mm.	
8.00–10.00*	1.0	25	50		Dog had been kept from food and water for 24 hours previous to experiment
12.00	7.0	67	85	2.0	
2.00	8.0	67	85	1.75	
4.00	7.0	62	80	2.25	
6.00	8.0	67	87	2.0	
8.00	7.5	65	85	2.0	

---

* Continuous secretion taken from 8.00 to 10.00 a.m.

TABLE 9

Gastric stimulation by gastrin at two hour intervals

1 cc. GASTRIN PROCEDURE	GASTRIC JUICE: DOG II				REMARKS
	Amount	Free acidity	Total acidity	Pepsin	
	cc.			mm.	
8.00–10.00†	3.0	57	25		Acidity lower than normally Dog had been kept from food and water for 24 hours previous to experiment
12.00	18.0	87	117	3.0	
2.00	18.0	90	110	2.5	
4.00	17.5	90	105	2.75	
6.00	19	97	117	3.5	
8.00	21	90	110	2.75	
10.00	19	87	105	3.0	
12.00	20	87	105	2.75	
2.00 a.m.	17	87	102	2.25	
4.00	17	82	100	2.25	
6.00	19	85	110	2.5	
8.00	16	70	100		Juice bloody (digestion of skin about pouch)
10.00	17	75	102		Juice bloody (digestion of skin about pouch)
12.00	16	72	100		Juice bloody (digestion of skin about pouch)
Meal* 12.00–1.00	10	70	90		Juice bloody (digestion of skin about pouch)

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* Normally the first hour of the meal produced 12 cc. of juice with free acidity of 90 and a total acidity of 115. The dog appeared to be very tired and did not eat with her normal degree of appetite.

†Continuous secretion.

Tables 8 and 9 are interpreted to mean that there is no fatigue of the gastric glands when stimulated by gastrin over a period of 26 hours. The slight reduction shown in table 9 is not significant because of the bleeding and the decrease in the acidity with the meal as compared with the normal is easily accounted for by the lack of relish of the food which was very manifest. This experiment is to be extended further. From the data obtained up to the present time the conclusion is warranted that the gastric glands are not fatigued by the continuous stimulation of gastrin during a period of 24 hours. Such a positive result is especially significant as during this period 240 cc. of juice were secreted by this method of chemical stimulation, although the dog had not had any water for 48 hours and had been kept in the stock during the period of collection.

GENERAL DISCUSSION

The question arises as to how this stimulation by water is produced. That the stimulation is not due to "the prolonged and widespread contact" of water with the gastric mucosa, as suggested by Pavlov, is shown by the stimulation occurring when the water is in contact with the mucosa only 1½ minute. Without deliberating one might explain by Pavlov's theory the results given in table 6, in which those stomachs that emptied water slowly gave the greatest response to stimulation by water. But it seems that the facts, as observed in this work, support the theory advanced by Carlson, Orr and Brinkman (8) who suggest that "the water washes traces of gastric secretogogues into the intestine, where they are absorbed and act on the gastric glands via the blood." For example, when water was put into the stomach and pumped out simultaneously 5 to 10 cc. were always lost, which could only be accounted for as having been emptied into the intestine along with gastric secretogogues from the stomach. The latent period of 5 to 10 minutes also suggests that such a mechanism exists. The observations made upon the relation of the emptying time of the stomach for water and gastric stimulation by water are also explained by this theory. For one would expect the secretogogues to be present in a higher concentration in the slow emptying than in the fast emptying stomach, as in the former there would be more time for digestion of mucin, food-remnants and of the proteins of the gastric juice and mucosa. Hence one would expect a greater stimulation by water in the slow emptying than in the fast emptying stomach. The fact that fatigue of the gastric glands, when stimulated by water, cannot be demonstrated is due to the formation and collection of secretogogues in the rugae of the stomach, which are washed out by the successive ingestions of water, these ingestions of water being far enough apart to allow the formation of secretogogues in the meantime. The glands not being fatigued by gastrin, they would not be fatigued by secretogogues.

The dilution of the blood, as has been suggested, cannot be a basic factor in gastric stimulation by water for stimulation results, as shown by experiments on the latent period, when practically no water (not more than 10 cc.) is absorbed.

Whether or not the drinking of 400 cc. of water could be substituted for the Ewald meal in practice is an open question and will continue to be until a large series of observations has been made upon both pathological and normal individuals. According to the series presented in this paper, one would be led to believe that the Ewald meal cannot be substituted by water, because those individuals that responded but poorly to stimulation by water gave almost the normal response to an Ewald meal in every instance.

Further it is apparent from the curves shown in this paper that in all studies of gastric secretion, normal or pathological, experimental or clinical, the continuous secretion must be studied and taken into account. The stomach contents, or residuum, is not a true index of the continuous secretion.

One is not surprised to find marked discrepancies in the literature upon the emptying time of the stomach for both food and water when the marked normal individual variations are considered along with the many factors, both psychic and constitutional, that influence the activity and functioning of the organ. And it is evident from the observations made upon the twenty men in this series that the statements published in textbooks of physiology on the very rapid rate of the emptying of water from the stomach (500 cc. in 15 minutes) are misleading and false.

As to gastric glandular fatigue it is only reasonable to believe that as long as the normal gastric glands are supplied by the blood with the normal quality and quantity of constituents that form the gastric juice no fatigue will be observed, but as soon as the blood fails to supply these necessary constituents a change in the normal character of the gastric juice will occur. Even then it cannot be said that the glands are fatigued, but have only been deprived of the raw products necessary for their normal functioning. The results of the experiments on the question of gastric glandular fatigue seem to bear out this idea. And it only remains to ascertain how long the secretion can be continued before the necessary constituents in the blood for the formation of gastric juice are depleted enough to produce a change in the normal character of the gastric juice, no food or water being supplied to prevent the depletion.

CONCLUSIONS

1. The ingestion of water with the meals (400 to 800 cc.) increases the amount and the free and total acidity of the gastric juice.
2. The ingestion of water with the meals decreases the emptying time of the stomach, due to the dilution of the stomach contents.
3. Food in the stomach retards the evacuation of water.
4. The emptying time of water from the normal human stomach varies, conservatively, from 400 cc. to 100 cc. in 15 minutes.
5. The manner of the discharge of water from the dog's stomach is, according to the observations upon four dogs, rhythmic and could very possibly correspond to peristaltic waves.[5]
6. All stomachs do not respond to stimulation by water, there being a marked variation in different individuals. Those stomachs that empty water slowly (150 cc. or less in 15 minutes when 400 cc. are drunk) respond much more than those that empty water fast. From the observations in this study water cannot be substituted for the Ewald meal.
7. The latent period of the gastric glands of man when stimulated by water is from 5 to 7 minutes.
8. It was impossible to demonstrate a fatigue of the gastric glands when stimulated by water or by gastrin for a period of 10 to 26 hours.

The writer desires to express his indebtedness to Doctors Luckhardt and Carlson for their valuable suggestions.

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[5] In a personal communication, Dr. A. B. Luckhardt stated that this observation corroborates his findings upon which he has more definite data to be published later and which is referred to in a recent article by Dr. Carlson in this Journal, xlv, 87.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.
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EXPERIMENTS ON THE NATURE OF THE SENSE OF SMELL IN THE COMMON CATFISH, AMIURUS NEBULOSUS (LESUEUR)

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1. INTRODUCTION

The question as to whether fishes possess a sense of smell separate and distinct from that of taste has for several species been answered in the affirmative (Parker, '10, '11, '13; Sheldon, '11; Parker and Sheldon, '12). Experiments on the common freshwater cat fish, Amiurus nebulosus, have been especially successful, and for this reason and because of its availability and comparative hardihood in the laboratory, this species was chosen for the work the results of which are given in this paper. My acknowledgments are due Dr. G. H. Parker for suggesting the problem and for helpful criticism throughout the work.

2. MATERIAL AND METHOD

The younger fish were found to be best for experimental purposes since they became accustomed to laboratory conditions more quickly and gave more uniform reactions than the older ones. It was discovered early in the experiments that a single specimen of Amiurus in a tank does not respond normally, if at all, to olfactory stimuli but that at least two individuals must be kept together. This gregariousness, which is so characteristic of the species, has already been commented on by Parker ('10) and by Parker and van Heusen ('17a, b). It takes generally some three weeks of residence in the laboratory before the fish become accustomed to the frequent changing of water, the presence of the investigator, etc., and since these experiments were concerned with the reactions of Amiurus to food-materials, the fish had to be brought into a receptive state toward food and were therefore left unfed during this period of readjustment. As soon as they nibbled eagerly at a bag containing minced earthworms, experimentation could proceed. The fish were kept, in some cases as long as five months, in good physical and experimental condition by feeding each individual daily half of a living earthworm.

The method chiefly employed in these experiments was the same as that of Parker ('10, '11) and of Parker and Sheldon ('12), viz., the suspending—in a tank containing the fish—of cheesecloth bags in which were concealed the materials to be tested. In a few cases the bags were attached to wires hanging from a wooden frame but after the discovery of the exceeding sensitivity of Amiurus to metal rods (Parker and van Heusen, '17a), cotton strings were employed in their place. In every trial two bags were used, one a dummy, containing merely a wad of cotton weighted with a small stone, the other the test-bag, exactly similar in appearance to the dummy, but containing some substance to be tested wrapped within the cotton wad. Rarely did the fish bite the dummy (cf. Parker, '11), yet in a few cases thos individuals which had been subjected to experimentation for several months would, during a trial, give two or three nibbles on the dummy, but this occurred only when their barbels touched the dummy, the fishes having been roused to swim by a stimulating substance in the test-bag, which they had not as yet located. They evidently learned to associate olfactory stimulation with the presence of a bag containing food and whenever they discovered any such bag by means of the sense of touch in their barbels, they bit at it. A very few bites on the dummy were sufficient, for the fish would then resume their search until they found the test-bag and once having found it, the numerous bites and often vigorous tussle left no doubt in the mind of the observer that, from the standpoint of olfactory stimulation, the few nibbles on the dummy-bag might be disregarded. If, however, the test-bag received no more attention than the dummy, viz., an occasional feeble bite, by a long trained fish, this was taken as proof that the substance in the test-bag was non-stimulating.

Further instances of the ability of Amiurus to learn were afforded by several specimens which would come to the surface of the water to receive bits of worm, etc., from the forceps or fingers, when the investigator waved his hand over the water. These fish would finally nibble at anything presented to them at the surface of the water, bare forceps, finger, paper, even pebbles, but when dummy and test-bags were placed on the bottom of the tank and the disturbance due to the lowering of the bags had ceased, the fish bit at the test-bag only when it contained some substance which proved to be also stimulating to other fish, and paid no attention to the dummy.

In some cases, e.g., with blood, the test-bag became colored and therefore different in appearance from the dummy; but upon observation it was quite evident that sight plays little part in the food reaction of Amiurus (cf. Parker and Sheldon, '12). Herrick ('02) found that catfish did not "remember the color of cotton," and a small piece of brick, the color of raw meat, failed to attract them though they bit eagerly upon it after it had been soaked in meat-juice. In the present experiments most conclusive evidence upon this point was afforded by the behavior of blinded fish (eyes totally removed). These blind fish appeared to become aware of the presence of food even more quickly than the normal fish, perhaps because their unceasing restlessness kept them awake, as it were, and receptive to stimulation while the normal fish at rest—the condition at the beginning of each experiment—were rather sluggish and required to be "awakened" by the stimulus. The blind fish located food in practically the same time as the normal fish. It is well known that Amiurus is more active at night than in the day (Parker and van Heusen, '17b), being in nature a night feeder (Herrick, '02).

A test-bag was never used for more than one material, a new bag and fresh cotton being substituted at each trial. If a material proved to be stimulating to a fish, the water in the aquarium was changed after the trial, the aquarium scrubbed and rinsed and another trial was not made until at least two hours had elapsed.

The two bags were let down as gently as possible into the tank while the fish were at rest. This could generally be accomplished without disturbing the fish further than momentarily decreasing their rate of respiration. When a test-bag containing a stimulating substance, such as minced earthworms, was placed some eight inches from the head of a fish, the following sequence of reactions usually occurred: (1) The respiration rate returned to normal after about one minute, i.e., to what it was before the disturbance created by the lowering of the bags. (2) After remaining normal for about two minutes, the rate of respiration increased. (3) The large barbels at the corners of the mouth would then begin to twitch, (4) and after a very few seconds the fish would give a huge gulp, (5) lunge forward, apparently spasmodic, and (6) finally swim about searching along the bottom of the tank. If the dummy-bag alone was placed in the tank there was no such behavior, the fish often remaining motionless for ten, fifteen or sometimes thirty minutes. The time elapsing between the lowering of the bags and the swimming of the fish varied greatly in the different trials and the variation seemed to be due as often to the individuality of the fish as to the nature of the substance tested. Substances only slightly soluble, such as dry blood albumin, did take longer to reach the fish, though when this particular material was previously soaked in water and the solution absorbed by the cotton used, the reaction time was the same as for other substances, viz., four to five minutes.

If, during the first fifteen minutes after introducing the bags into the tank, the fish reacted to the material in the test-bag by nibbling, nosing the bag or searching vigorously in its vicinity, the position of the two bags was reversed and observations were taken for another fifteen minute period. If, however, there was no reaction during the first fifteen minutes, the bags were left undisturbed for fully thirty minutes. Almost never did a trained fish fail to respond to a simulating substance in less than ten minutes, so that after the experimenter had learned the characteristics of a particular fish, fifteen minutes was often enough to indicate the efficiency of a substance as a stimulating agent. For purposes of comparison frequent trials were made with bags containing minced worm and unless the fish reacted readily and vigorously to these the previous sets of experiments were discarded.

A second form of experiment, employed with liquid substances, confirmed the results of experiments with bags. Two fish were placed in shallow dishes of water and when they had become perfectly quiet a pipetteful of the liquid to be tested was gently released above their anterior nasal apertures. If worm-juice or other stimulating material was used, the same series of reactions took place as with the bags, viz., increase in rate of respiration, twitching of the large barbels, a gulp and a lunge forward, but all these reactions occurred in very rapid succession. When a non-stimulating substance, such as water, was used, the fish usually showed irregularity in their rate of respiration but no other response followed. If the anterior nasal apertures of the fish were closed by sewing (Parker, '11), the only reaction, even to worm-juice, was a slight change in the respiration, and in many trials even this was not observable.

Table 1 gives a list of substances used in preliminary tests and their relative values as stimulating agents. To determine these values a record was taken of the number of bites and vigorous nosings made by two fish during thirty minutes. To that substance which caused the greatest number of bites was assigned the value 100, and the others estimated accordingly. Since some thirty different pairs of fish were used in the experiments, at different seasons of the year, and, as indicated above, each fish seems to possess more or less of an individuality of its own, and all could certainly not have been in exactly the same physiological state as regards food, several of the values calculated on the basis indicated have been slightly changed in order to make the relative values consistent throughout all the tables.

TABLE 1

Effectiveness of various materials as olfactory stimuli to Amiurus nebulosus—based on the number of bites made by two fish during half-hour trials. The most stimulating materials are graded 100 and the others in proportion

WORMS	
A Lumbricus	
Whole	90
Minced	100
Decayed	100
Rotten	0
Dried	90
B Eisenia	
Minced	80
Decayed	80
BEEF LIVER	
Fresh	90
Stale	100
LEAN BEEF	
Fresh	20
Stale	60
Decayed	0
Dried	0
Stero-cubes	0
Commercial peptone	0
BLOOD	
Frog	20
Human	30
Sheep	60
Beef (fresh)	60
Beef (decayed)	0
Worm	75
MUTTON	
Fresh	10
Decayed	0
FROG MUSCLE	
Fresh	0
Decayed	0
AMINES	
Prophylamine	0
Higher amines (1)	0
Higher amines (2)	0
FISH MUSCLE	
Amiurus, fresh	0
Amiurus, decayed	0
Herring	0
Mackerel	0
SLIMY SUBSTANCES	
Frog's eggs	20
Limax	15
Necturus' slime	15
Human saliva	40

3. GENERAL RESULTS

It has been intimated that this fish, Amiurus nebulosus, is more of less of a scavenger and it is a tradition, at least among rural fishermen, that rankly smelling substances, such as badly decayed meat or worms, are even better bait than fresh meat and fresh worms. Dean ('91) remarks that Amiurus nebulosus is an objectionable neighbor since fish eggs are often found in its stomach. "Nor is it fastidious in its diet, 'from an angleworm to a piece of tin tomato can', it bolts them all. . . . Professor Goode has already noted the attractiveness of salt mackerel for herring bait." Forbes and Richardson ('08) found that the food of this fish consisted of "small bivalve mollusks, larvae of insects, distillery slops and accidental rubbish." One specimen contained eighteen leeches.

An examination of table 1 will show that the results of my experiments rather disagree with the idea of "un-fastidiousness" in Amiurus nebulosus in its choice of food. With the exception of decaying earthworms, which will be discussed later, no putrefying material proved at all attractive to the fish nor was I successful in getting bites or even nosings upon bags soaked in several of the lower amines, though they most decidedly possess the characteristic odor of decaying meat.

The difference in behavior between catfish in the laboratory and those in a pond or river is readily accounted for when one realizes that an olfactory stimulus renders the fish aware of food and releases the impulse to seek for it, but that the food itself is usually located by the sense of touch (and perhaps taste) in the barbels after contact with it. In the tank there were only two cloth bags to interrupt the smooth glass surface, but in the natural habitat of the fish there are all sorts of objects over which the barbels are dragged. A very "hungry" fish will often nose quite inedible things, such as a pebble, and in one case I observed a fish, which had been roused to the seeking reaction by a bag of minced worms, take into its mouth its own faeces, only to cast them out immediately. So in nature hungry fish may, during their restless movements at night, swallow many things, e.g., salt mackerel and herring bait or even a bit of tin can, which do not stimulate the sense of olfaction.

But since human saliva proved to be fairly stimulating to the fish, the time-honored custom of "spitting on one's bait" does seem to be more than superstition, and perhaps for this reason may receive the sanction of science.

Among the substances tested, earthworms, beef liver and blood seemed to be the most promising for study.

4. THE COMPONENTS OF EARTHWORM MATERIAL AS STIMULATION AGENTS

It was rather astonishing to find that a living, uninjured earthworm, Lumbricus terrestris, although carefully wrapped in a wad of cotton and so tied in a cheesecloth bag that no movement was discernible, should be discovered as quickly by Amiurus and should prove to be practically as stimulating as small pieces of chopped fresh worm from which the blood and body-juices could readily escape. After the trials the worms were carefully examined and though the bags had been bitten, often as many as thirty times, the worms were found to be uninjured and their epidermis intact. The slime thrown off by Lumbricus as it travels unmolested over and through moist cotton is only half as stimulating as the worm itself but if a specimen on cotton is prodded and mauled, though not so severely as to injure its epidermis, this slime is equal in its stimulating power to the worm itself. It was noticed that when the worms were thrown into water of the same temperature as that in which the fish were kept, they writhed about much like the prodded worms on cotton. It seemed therefore that the ordinary products of the slime glands were not responsible for this attraction of the fish for whole worms, but that some further secretion, perhaps fluids from within the body-cavity escaping through the dorsal pores, were the stimulating agents. A quantity of ropy slime sufficient for experimental purposes was thrown off by living worms placed in water at 28° to 30°C. or in dilute (10 per cent) alcohol. Such slime, both before and after filtering, is exceedingly stimulating to the fish. Nevertheless, when this fact is taken into consideration with the results of trials on human saliva, Necturus slime, Limax and frog's eggs, one might naturally infer that the source of stimulation in these materials is that chemical substance common to them al', mucin.

The glycoprotein, mucin, is a relatively stable protein, not coagulated by heat, soluble in dilute alkali and precipitated by dilute acid. Slime from one hundred living earthworms in 250 cc. of water kept at 30°C. for thirty minutes, after being filtered, twice precipitated, washed and dissolved, and again precipitated and washed, yields a very small amount of grey slimy mucin, which when dried can be ground to a dirty yellow powder (cf. Hammarsten, '14, p. 170). This dried mucin does not dissolve in tap-water, and is not stimulating to Amiurus in this state. But even when dissolved in 1/1000 M KOH (an OH concentration which is not stimulating to Amiurus), it caused only the slightest reaction on the part of a few fish and none at all on the majority of those tested. Mucin prepared from human saliva (Hawk, '14, p. 123) or from the salivary glands of sheep, either dry or dissolved, failed to attract the fish. If sheep mucin is washed free from acetic acid after precipitation and immediately smeared on the outside of a bag, the fish will nibble at such a preparation but only when their barbels come into direct contact with the slimy material.

TABLE 2

Effectiveness of preparations from worms (Lumbricus terrestris) as olfactory stimuli to Amiurus

1. Whole worms	
Living, uninjured	90
Dried	100
2. Worm slime	
Cotton traveled over by worms	50
Cotton traveled over by prodded worms	85
Slime from worms in water at at 30°C. or in 10 per cent alcohol	95
Ether extract	0
Ether residue	85
Chloroform extract	0
Chloroform residue	25
Heated to 60°C	60
Heated to 70°C	0
Open to air four days	0
Preserved one week with thymol	40
Mucin	5
3. Minced fresh living worms	
Entire product	100
Filtrate	100
Residue from filtrate fresh	100
Same dried	100
4. Red filtrate from fresh minced worms	
Albumins	25
Globulins	25
Peptones	25
All proteins precipitated by acetone	100
Acetone residue from above	60
5. Ether extraction	
Fat	25
Residue fresh	100
Residue dried for months	100
6. Boiled	
Liquor	100
Acetone precipitate from liquor	100
Acetone residue from above	80
Worm bodies	100
Worm bodies dried	100
7. Decayed	
Without treatment	100
Alcohol extract	0
Alcohol residue	90

It is evident, therefore, that mucin is not the chemical substance which causes Amiurus to bite upon bags in which are concealed whole, living worms; mucin does, however, seem to stimulate the barbels when they come into direct contact with it.

There are indications that the substance or substances in the filtered slime of Lumbricus which attract Amiurus are of the nature of proteins.

(1) If the slime is shaken with ether, the two layers separated and the ether evaporated from each layer before an electric fan, the ether extract (on filter paper) has no attraction for the fish and the water residue loses some of its stimulating power. If the slime is similarly treated with chloroform, not only does the chloroform extract not stimulate the fish but the water residue becomes much less stimulating. If the slime is boiled or even heated to 70 °C. it loses its stimulating power. Heating to 60°C. for three minutes slightly impairs this stimulating power but heating to 50° or less has no effect. Ether, chloroform and heat are general coagulating agents for proteins (Hammarsten, '14, pp. 97, 107). (2) If the slime is allowed to remain open to the air for several days and putrefaction takes place, it becomes non-stimulating. When preserved with a few drops of thymol solution, it retains somewhat of its stimulating power. Yet the ordinary chemical tests for proteins, Biuret, Millon's, xanthoproteic, etc., applied after the removal of mucin give only negative results. This probably means that the concentration of these substances is so low that they can not be detected by the ordinary color tests. If this is true, one of the characteristics of the olfactory sense of Amiurus is its ability to respond to exceedingly small amounts of substance, and this is in accord with what we know of the human sense of smell (Parker, '12).

Proof that the sense of smell alone is concerned in the reactions of Amiurus to worm slime is afforded by the behavior of fish with their barbels removed, and therefore lacking the majority of their gustatory organs (Parker, '10). These barbel-less fish find bags soaked in the slime only slightly less readily than normal fish. And in addition, fish with their olfactory apparatus temporarily eliminated by the sewing of their anterior nasal apertures, fail to pay any attention whatsoever to such a bag. A slight exception to this behavior occurs when an operated fish which has been subjected to experimentation for several months happens in its movements to touch the bag. Such a fish may give one or two gentle nibbles upon the bag. This is evidently nothing more than a matter of learning as previously described. or the response of a very "hungry" fish to tactile stimulus.

When living worms are passed through a meat-grinder and then allowed to soak in water for twenty-four hours, using thymol as a preservative (Wodehouse and Olmsted, '17), a clear red solution is obtained, which is as stimulating to Amiurus as the pieces of worm themselves. Upon separating these water-soluble proteins into globulins, albumins, peptones, etc., somewhat according to the scheme outlined by Hawk ('14, p. 123), there were obtained preparations which, in either the moist or dry state, were always much less attractive to the fish than the original solution. The same was true of proteins soluble in salt solution. The insoluble residues from the water or salt extraction were always stimulating in either the moist or dry state. If, however, the red water-solution was filtered off immediately after grinding the worms and the proteins precipitated as a whole by acetone, this aggregate of proteins was very stimulating to the fish. But any attempts to separate them by redissolving, partially and wholly, saturating with ammonium sulphate, dialysing, etc., reduced their effectiveness almost to zero.

It was stated above that if the slime from Lumbricus was boiled or even heated to 70°C. it lost its attractiveness for Amiurus. This is not true of the worms themselves. Both the worms and the liquor in which they are boiled are as stimulating as fresh raw worms. If such boiled worms are dried, they lose none of their stimulating power even after six months.

When the proteins in the liquor from boiled worms are precipitated by acteone, a slimy grey coagulum is obtained, which is very stimulating to the fish, and even the acetone filtrate, after being evaporated to dryness on the steam-bath, is only slightly less so. If, however, alcohol is used as the precipitating agent, the coagulated proteins are only slightly stimulating while the alcohol filtrate is as strongly stimulating as the original solution. Likewise an alcohol solution made by placing living worms in 95 per cent alcohol until they become hard and brittle, is exceedingly stimulating, but an alcoholic solution made from decayed worms is non-stimulating though the residue of worm bodies, having lost much of its disagreeable odor, is stimulating. Chemical tests on the alcoholic solutions which prove to be stimulating indicate, but only very faintly indeed, the presence of a peptone. But doubt is thrown on the assumption that this is really the chemical substance responsible for stimulation since worm peptone prepared by several methods was hardly at all stimulating. This may again be interpreted as indicating the presence of exceedingly small amounts of stimulating substances, perhaps of the nature of proteins (since they seem to be destroyed by putrefaction), but in this case non-coagulable by heat.

If living worms are allowed to dry without putrefaction, they are found to be exceedingly stimulating to the fish, even after a year in this condition. The fat of freshly dried worms, extracted with ether in a Soxlett apparatus, is yellow and closely resembles ordinary animal fat in appearance and properties. Such worm fat is only slightly attractive to Amiurus, showing that the very stimulating acetone and alcohol soluble substances mentioned above are not fats, and this is further supported by the fact that the hard dry pieces of worm remaining after the ether extraction are exceedingly stimulating.

Proof that these substances are not of the nature of volatile etherial oils is given in the results of the following experiments. Freshly ground living worms were placed with water in a flask provided with two openings. Through one of these openings air was forced up through the mixture and out through the other into a tube with a capillary outlet. This outlet was situated at the bottom of a cylinder of distilled water cooled by ice. Although air was bubbled through the worm mixture for twenty-four hours, the cooled water did not take on any peculiar or distinctive odor and such water was non-stimulating to the fish. A similar procedure when carried out on decaying worms did impart to the cooled water the characteristic odor which accompanies putrefaction, but this liquid was likewise non-stimulating. In one case a bag of minced earth-worms which had been left in running water for over a week without a preservative developed an almost intolerable odor and in this condition had no attraction whatsoever for the fish. Very completely decayed worms from which the water soluble substances have been practically entirely removed are therefore non-stimulating.

These results show that it is not the decayed material—the broken-down proteins—which stimulates Amiurus, and had the so-called "decayed" worms been thoroughly putrefied, they would probably have failed to excite the fish. In other words, the stimulus which caused Amiurus to react to "decayed" worms came from the still undecayed, unchanged substances.

Both fresh and decayed Eisenia foetida were stimulating to Amiurus, but some ten minutes after the fish bite these bags, they appear to be both attracted and repelled by the dung-worms. In a short time the repulsion becomes stronger than the attraction, the fish, on coming into the immediate neighborhood of the bag, dash wildly about the aquarium, jerk their heads away and often back off most vigorously. For this reason no extended experiments were carried out with Eisenia material.

5. EXPERIMENTS WITH BEEF LIVER AND BLOOD

The similarity of the results of experiments with earthworms, beef liver and blood will be evident upon comparing tables 2 3 and 4. It will be noted that in no case was there found any component of the original protein mass that was not decidedly less stimulating than the original combination.

Fat was only very slightly stimulating.

If the watery extract from fresh liver is filtered and allowed to remain over night, a heavy brown coagulum settles out. This coagulum is slightly more stimulating even than the original water extract and possesses the odor of stale liver, an odor quite distinguishable from that of fresh liver. From table 1 it will be seen that stale liver is slightly more stimulating than fresh. Nevertheless ice-cooled water through which air was bubbled after having been previously forced through ground stale liver, and which possessed most distinctly the odor of stale liver, utterly failed to stimulate the fish. It seems evident, therefore, that the substance which gives stale liver and this coagulum from the watery extract of fresh liver the characteristic odor which distinguishes them so markedly from fresh liver, is not the same substance that renders them more attractive to Amiurus than fresh liver.

TABLE 3

Effectiveness of preparations from beef liver as olfactory stimuli to Amiurus

MINCED LIVER		FILTRATE FROM FRESH MINCED LIVER	
Fresh	90	Albumins and globulins together	30
Stale	100	Globulin	20
Filtrate from fresh	80	Albumin	15
Coagulum from fresh filtrate	100	Peptone	0
Dried coagulum	20	All proteins precipitated by acetone	0
Dried coagulum redissolved in water	60		
ETHER EXTRACTION		**BOILED LIVER**	
Fat	0	Liquor	100
Residue dry	80	Residue	90

A further instance of substances possessing a very similar odor to the human sense of smell but differing markedly in their effect on the fish, is shown in the residue after ether extraction of stale beef or commercial dried beef. Only the former of these two products stimulates Amiurus to bite.

The most striking differences in the results of the experiments on earthworms, beef liver and blood are: (1) Of the three kinds of material, earthworm alone was stimulating when "decayed,"—but other experiments show that the products of putrefaction are not the stimulating agents, (2) earthworm proteins alone retain their stimulating power after having been precipitated by acetone. This causes one to doubt whether such proteins (the globulins, albumins, peptones, etc., which can be detected and separated from each other by the ordinary chemical methods, and might be called the "gross" proteins) are really, even in the case of the earthworm, the stimulating agents for olfaction in Amiurus. Is it not more probable that in all these materials, substances which are present as "chemical traces" only, are responsible for the reactions of the fish? Some of them were shown to be destroyed by heat, others not; some are soluble in acetone and alcohol, others are rendered inactive by these reagents; they are not of the nature of fats or volatile oils; nor are they the products of the decomposition of proteins, in fact putrefaction seems to destroy them. In view of these characteristics it seems possible that they may be of the nature of proteins themselves.

TABLE 4

Effectiveness of preparations from beef blood as olfactory stimuli to Amiurus

BLOOD AS WHOLE		DEFIBRINATED PLASMA	
Fresh	60	All proteins precipitated by acetone	0
Decayed	0	Albumins	0
Fibrin	0	Globulins	0
Defibrinated plasma	60	Peptones	0
		Ether extract	0
		Ether residue	0

The responses of Amiurus to stale liver and to the residue after extraction with ether were shown to be due to olfactory stimulation alone since fish with anterior nasal apertures sewn (with the exception of long trained fish) always failed to find bags of such material, while if the stitches were cut and the threads still left in the skin, the fish found the bags as quickly and bit on them practically as often as before the operation.

6. EXPERIMENTS WITH CHEESE

Fishermen have informed me that cheese, especially Limburger, is excellent bait for catfish. Table 5 gives the results of experiments on the effectiveness of this commodity as a stimulating agent, and shows that even Limburger cheese is not especially stimulating.

TABLE 5

Effectiveness of cheese as olfactory stimulus to Amiurus

AMERICAN		LIMBURGER	
Fresh	0	Fresh	10
Stale	0	Ether extract (rancid fat)	5
		Ether residue (protein)	5

7. THE ACTION OF THE BARBELS

A trained fish with its anterior nasal apertures sewn does not respond to bags of minced worm gently lowered in front of it but if the bag is carefully drawn toward the fish, the moment it touches a barbel, it will be seized by the fish. Rarely does this occur with a dummy-bag and never with a glass rod. This, again, seems to be a matter of learning, in which the sense of touch is concerned. The fish learn to distinguish the "feel" of the bag, which so often contains food. That the barbels do render service in food-getting is shown by the records of three pairs of fish whose reactions were followed for several months. From one fish of each pair all the barbels were removed and almost without exception this fish bit fewer times than its mate during the seventy odd half-hour trials. It was roused to action by an olfactory stimulus as soon as the other but was less able to locate the source. Parker ('10) describes the behavior of such fish as follows:

> Those without barbels, but with their olfactory apparatus intact, almost always made several sharp turns when near the wad (of minced worms) as though seeking something, and either moved slowly away, or swam more or less directly to the wad and began to nose and nibble it.

When a normal catfish receives an olfactory stimulus, it swims about with its barbels dragging along the bottom of the tank. The fish almost invariably stops for a moment if its barbels touch any object. If this object is edible the fish may nibble or swallow it, but if inedible the fish, after nosing the object, usually resumes its interrupted search. It appears, therefore, that the barbels are very valuable to Amiurus in procuring its food but it receives stimuli through these organs only when they are in contact with a relatively large amount of material (cf. Herrick, '02, p. 257). The small particles of (presumably) molecular dimensions diffusing through water from a bag of minced worms cause no reaction in a fish whose olfactory organs are incapacitated to receive such stimuli. But if the barbels of such a fish come into contact with the bag, the fish *may* give a bite. If, in addition to the sense of touch, that of taste is involved in this reaction,—and Parker ('08) has shown that Amiurus possesses a gustatory sense located in its barbels,—then this sense in Amiurus is comparable to the human sense of taste (Parker '13), since a relatively large amount of substance locally applied is necessary to stimulate our organs of taste.

SUMMARY

1. Amiurus nebulosus bites readily on bags of earthworm, beef liver and blood.

2. The responses to these substances arise from the stimulation of the olfactory organs, as is proven by lack of responses when the olfactory apparatus is eliminated, and by the facts that blinded fish find this food as readily as normal fish, and barbel-less fish only slightly less so.

3. The barbels are of value in finding food only by coming into direct contact with it.

4. Decayed animal material is not attractive to these fish.

5. Although earthworm slime, human saliva and similar substances do attract Amiurus, the mucin which they contain is not a stimulating agent for the olfactory organs, though it may be so for the barbels.

6. All attempts to separate the proteins of earthworm, liver or blood resulted in preparations less stimulating than the original combination.

7. The fats from these materials were practically non-stimulating but the ether residues were decidedly stimulating.

8. It is suggested that the substances which stimulate olfaction in Amiurus are possibly of a protein nature since they are destroyed by putrefaction; they are not the products of protein decomposition; some of them are rendered ineffective by heat or addition of alcohol or acetone, others not; they are not of the nature of fats or etherial, volatile oils; and they are present in such small quantities that they are describable as "chemical traces," and can not be detected by the ordinary qualitative tests for proteins.

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THE RELATION OF THE DORSAL ROOTS OF THE SPINAL NERVES AND THE MESENCEPHALON TO THE CONTROL OF THE RESPIRATORY MOVEMENTS

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The general history of the work upon the nervous mechanism of respiration begins with the experiments of LeGallois and Flourens in the early part of the nineteenth century. In 1811 LeGallois (1) demonstrated that after transection below the medulla, all respiratory movements of the body cease with the exception of movements of the mouth, which cease also after section of the medulla. Flourens (2) amplified and confirmed this work (1842–1851). His experimental procedures began by removing the cerebrum, then the cerebellum, then the corpora quadrigemina of an animal (rabbits and pigeons were mostly used). Respiration persisted until sections of the medulla were made, then it failed. Reversing the operation and beginning with the lumbar spinal cord, making successive sections upward he found that

> In destroying the costal spinal cord, the rise and fall of the sides diminished gradually and when he had finished, had entirely disappeared.

As he continued to make sections upward, respiration was maintained, although with difficulty, by the diaphragm until the origin of the nerves of the diaphragm was reached when with their section and consequent cessation of the movements of the diaphragm, all effective respiration stopped, for the yawnings of the mouth and glottis which alone survived had no effect. He then proceeded in a reverse manner, removing the medulla by transverse sections from front to rear. The yawning movements disappeared first; then the dilation of the nostrils; the inspiratory movements of the trunk alone survived and finally these failed also. These experiments appear to indicate that the spinal respiratory nerves are unable of themselves to maintain rhythmic respiratory movements but are dependent on the action of a central coördinating mechanism situated somewhere above the lower end of the medulla oblongata.

Volkmann (1842), Longet (1847) and Schiff (1858) (3) showed that the central respiratory mechanism is a double organ which can be divided by a median longitudinal section without causing the death of the animal; and Longet, and more particularly Schiff, endeavored to show that this central mechanism is located in the nucleus of the grey matter in the alae cinereae in the lower part of the bulb, on each side beneath the floor of the fourth ventricle, and that the paths by which the impulse is conducted thence to the spinal cord run in the lateral bundles,

> Unilateral section of which at the lower level of the bulb, or at the level of the second or third cervical vertebrae, suffices to produce respiratory paralysis on the same side.

In opposition to this view, Brown-Sequard (4) enunciated his doctrine of "inhibitory centers." He showed in 1860 that if young animals were kept alive by artificial respiration for some time after section of the spinal cord below the medulla, when the artificial respiration was stopped, coördinated movements of the thorax and diaphragm might still be observed for a time. He therefore concluded that the center for respiration was not sharply localized in the medulla but extended throughout the spinal cord. The effects of section of the spinal cord below the medulla, he attributed to "inhibition" of these centers following the lesion of the cord, and he compared the phenomena with those of spinal shock.

This doctrine, including that of spinal respiration, was later presented in fuller form by Langendorff (1887) and Wertheimer (5) who observed that the respiratory muscles of the trunk could sometimes be made to contract after separation of the cord from the bulb in animals poisoned with strychnine, in animals with the cord artificially cooled or subjected to prolonged artificial respiration. Wertheimer declared that such contractions showed the power of the spinal cord to originate respiratory impulses.

Such an hypothesis has, however, been too often refuted to be at all acceptable at the present time. Schiff, in his early exposition of respiratory hemiplegia, demonstrated that section of the spinal cord at the level of the second and third cervical vertebrae paralyzes the respiratory mechanism.

Porter (6), in his study of the innervation of the diaphragm, followed a similar line of reasoning. He showed that since hemisections of the spinal cord above the phrenic nuclei do not inhibit the diaphragm on the same side, it follows that two hemisections altogether separating the phrenic nuclei from the bulb do not inhibit the diaphragm on their respective sides. In other words, the arrest of thoracic and diaphragmatic breathing in consequence of the separation of the phrenic nuclei from the bulb is not an inhibition. But one explanation of the arrest is then possible; the phrenic nuclei effect no respiratory discharge after their separation from the bulb because they receive no impulses and cannot originate them. Hence the cells for the discharge of respiratory impulses are situated above the calamus scriptorius and not in the spinal cord.

In addition to this, Starling (7) has pointed out that cells from which the nerve fibers go to the respiratory muscles can, like the motor cells of other parts of the organism, be affected by impulses reaching them along various paths. Their normal activity in respiration depends upon impulses from the medulla but they can also be affected along other tracts derived ultimately from the posterior roots, at the same or higher levels of the cord.

Hering (8) has concluded that after division of all the dorsal roots of the frog, the motor cells cannot discharge when removed from peripheral stimulation, and in the case of respiration, he is convinced that

> The normal rhythm of respiration is bound up with the integrity of the accompanying centripetal nerves.

Of late years, however, the relative importance of the dorsal roots of the spinal nerves in the maintenance of respiration has been overlooked; perhaps the refutation of the ideas of "spinal respiratory centers" had a discouraging effect, certain it is that little or no mention of the dorsal roots and their connection with respiration is made in present-day literature. The best pronouncement with which I am familiar has been made by Luciani (9) who thus epitomizes their activity:

> When the auto-regulation by means of the vagi is suppressed, an abnormal type of respiratory rhythm appears which, although it provides for a degree of pulmonary ventilation sufficient to maintain life, must yet be termed dyspneic since it is not obtained without useless expenditure of energy. Under these conditions it seems to us probable that a self-regulation comes into play due to the rythmical and alternate excitation of the sensory paths of the inspiratory and expiratory muscles.

The question has often arisen as to whether there is a mechanism for the integration of the respiratory movements higher than the medulla. The opinions of the various authors who have written upon this subject appear to be somewhat divided.

Starling (7), in citing the work of Rosenthal and Marckwald, states,

> In the rabbit, section through the upper part of the medulla oblongata, separating the respiratory center from the higher parts of the brain, is equally with out effect on the depth and rhythm of the respiratory movements. A great change is observed, however, if the vagi are subsequently divided under these conditions.

Nikolaides (10) says that in rabbits, isolation of the medulla oblongata from above causes almost the same effect as double vagotomy.

Luciani (9) states that

> When the brain is extirpated to the level of a plane which passes along the inferior limit of the pons, or when the section is made at the level of this plane, it will be seen that after temporary disturbance, the animal continues to breathe in a regular, perfectly coördinated manner.

H. Newell Martin (11) found that

> On stimulation of the mid-brain of the rabbit, close to the iter and beneath the corpora quadrigemina, there is a respiratory regulating center similar to that of the corpora bigemina of the frog.

Marckwald (5) found that on blocking off the respiratory center from the brain above by the injection of paraffin into the common carotid, if these higher paths are cut off, the respiration remains regular, although deep, and perhaps in the course of time tends to resume its original type; but if the vagi are also sectioned, the respiration is entirely changed; periods of rapid breathing alternate with periods of complete cessation until the animal dies.

From the literature here quoted it will be seen that division of the vagi, in connection with section at the level of the corpora quadrigemina has been considered. The possible relationship of the dorsal roots of the spinal nerves has, however, been given no attention. I have therefore performed a series of experiments with a view to determining whether there is a possibility of such a relationship.

These experiments have extended over more than a year, and include results upon about forty cats. These animals were first etherized and then tracheotomized and ether was given by means of a tracheal cannula. Tracings of both costal and abdominal respiration were taken by means of Crile stethographs attached to Verdin recording tambours. I will consider the results first of section of the dorsal roots of the spinal nerves alone, then at the level of the posterior corpora quadrigemina alone and finally the effects of the two operations together.

After a control tracing of normal respiration (under ether) was taken, laminectomy was done and the dorsal roots of the spinal nerves were then sectioned, sometimes in both thoracic and cervical regions and sometimes in the cervical region only. If the dorsal spinal roots are cut in the thoracic region alone there is a diminution of costal respiration although abdominal respiration remains unaltered and the rate is very little changed; if the cervical dorsal roots also are involved, independent costal respiration disappears, such costal respiration as is present being passive and induced by the abdominal respiration as the tracing of March 2, 1918 (fig. 1) shows. Such respiration is slower than normal but the general character of the respiratory curve is not altered. When the dorsal roots are cut in the cervical region alone, thoracic respiration is not greatly changed. An animal whose dorsal spinal roots have been divided aseptically may be kept alive for an indefinite period. Such an operation, indeed, is analogous to the condition found in some cases of tabes dorsalis in which the functions necessary for the maintenance of life may be performed adequately enough although precision of movement is lacking.

It is of interest to observe in connection with the experimental work the remarkable compensatory power of the individual dorsal roots. If, for example, in sectioning the dorsal spinal roots in the thoracic and cervical regions, a single root on either side be left intact, costal respiration remains much better than the general severity of the operation and the number of roots cut would lead one to suppose possible. A study of the nervous system impresses one more and more with its remarkable adaptive facility in the rearrangement of channels for the conduction of nervous impulses when the normal ones are cut off, and this is particularly exemplified in the conduct of the dorsal spinal roots of the thoracic region of the cord.

Following is a protocol of an experiment in which the dorsal roots were divided.

March 2, 1918. Male cat (fig. 1).
Ether, tracheotomy.
Laminectomy.
Control tracing of respiration taken (part 1).
Dorsal spinal roots cut from third cervical to lower thoracic.
Respiration tracing taken (part 2).

In this experiment, the significant factor is the complete cessation of an active form of costal respiration, such slight passive movements as are present being the results of the active diaphragmatic respiration. The rate, however, is not greatly altered.

It is evident that in the maintenance of respiration a central integrating mechanism is of first importance. We are well aware of the necessity of the integrity of the respiratory center in the medulla for the initiation of respiratory movements, but is there no mechanism for the integration of nervous impulses concerned with respiration higher than the medulla? In other words, if all portions of the brain above the medulla were removed, would respiration proceed in the same manner as before?

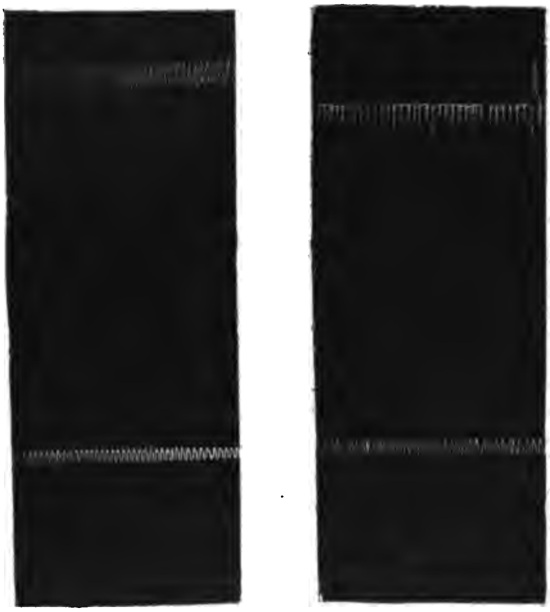

Fig. 1

Part 1. Respiration after laminectomy has been performed.

Part 2. Respiration after section of the dorsal roots of the spinal nerves from the third cervical to the lower thoracic. Upper tracing represents costal lower abdominal respiration.

In the technique for the operation of section of the brain stem above the medulla, the carotids were first tied off to prevent excessive hemorrhage and the animal was then either decerebrated by removing the hemispheres from the cranial cavity or a trephine opening was made over the occipital ridge and the corpora quadrigemina were sectioned through it.

I have found that sections in front of the corpora quadrigemina and between the anterior and posterior corpora produce no effect upon respiration; when, however, the section cuts into or behind the posterior corpora quadrigemina, there is a change in the character of the respiration. It appears to become slower and less regular than the normal type; still, it is hardly of a gasping character and maintains a very fair type of ventilation.

The difference in conduct between the results of this operation and that of complete section of the dorsal roots in the thoracic and cervical regions is one of degree rather than kind. In the latter case, a few channels for sensory impulses may remain above and below the sections—and we have mentioned the compensatory power of the dorsal roots in this respect—while the conduct in the former case implies a total lack of these sensory impulses.

In this connection, we cite the protocol of

March 16, 1918. Male cat (fig. 2).
Ether, tracheotomy.
2.00 p.m. Normal respiration (part 1).
2.45 p.m. Carotids tied off, then section behind corpora quadrigemina.
3.30 p.m. Good respiration, see tracing (part 2).

Such respiration as this gives no indication of the dyspnea which some authors have found and does, to some extent, resemble the slowing obtained after double vagotomy. I have observed at various times, however, that if after section is made there occurs a hemorrhage into the fourth ventricle, which causes a clot producing pressure upon its floor, then dyspnea always occurs. But if no such hemorrhage occurs, dyspnea is not present except in a very slight degree.

As I have previously indicated, the spinal cord has not been regarded as an important factor in respiration, during late years, and even when the possibility of spinal respiratory centers was under consideration few authors ever expressed the idea of a relationship between the dorsal roots and these spinal centers. It has been shown in a previous paper (15) that the dorsal roots undoubtedly play an important part in the sensory mechanism of costal respiration and that fibers concerned with afferent impulses pass up the spinal cord; our present work has served to confirm these findings and to extend them. Moreover, section of the brain stem at the level of the posterior corpora quadrigemina produces immediate and lasting effects upon the respiration. Since the mesencephalon contains afferent and efferent fibers from the spinal cord, the question presents itself as to possible relationships between the dorsal roots of the intercostals and the mesencephalon as shown by the effect of section at the level of the posterior corpora quadrigemina and the dorsal roots of the spinal nerves in the cervical-thoracic region. Following is a protocol of such an experiment.

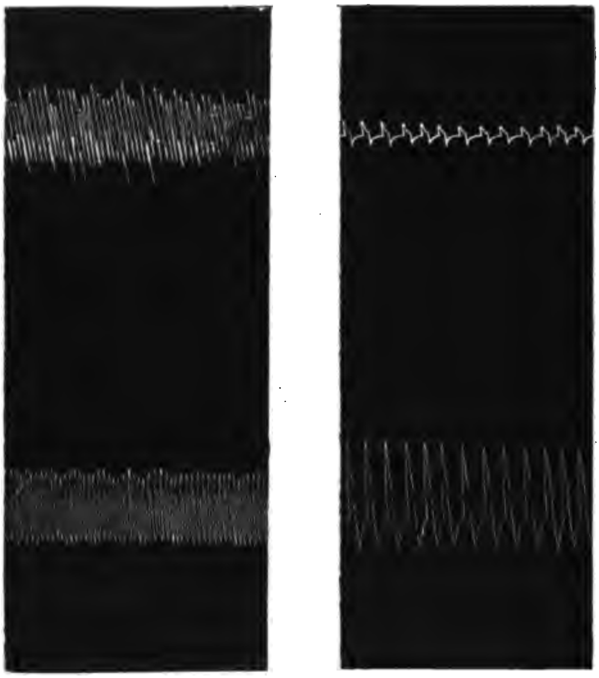

Fig. 2

Part 1. Normal respiration.

Part 2. Respiration after section behind the posterior corpora quadrigemina. Upper tracing represents costal, lower abdominal respiration.

July 13, 1917. Female cat (fig. 3).
Ether, tracheotomy, laminectomy (part 1).
2.40 p.m. Carotids tied off.
2.45 p.m. Decerebration.
2.55 p.m. Section behind the corpora quadrigemina (part 2).
3.20 p.m. Dorsal roots in cervical and upper thoracic regions cut (part 3).

From this experiment an interesting phenomenon may be observed, namely, that *after section of the posterior corpora quadrigemina, subsequent section of the dorsal roots is followed by no additional effects.* Such a finding leads one to conclude that certain of the sensory impulses at least, if not all connected with respiration from the dorsal roots of the intercostals, pass through the posterior corpora quadrigemina since the difference in severity between the results of the two operations is about the difference that might be expected between total and partial elimination of the afferent impulses from the dorsal roots. Moreover, the fact that section of the dorsal roots after section of the corpora quadrigemina produces no change in the character of respiration shows that the entire effect was obtained by the first division.

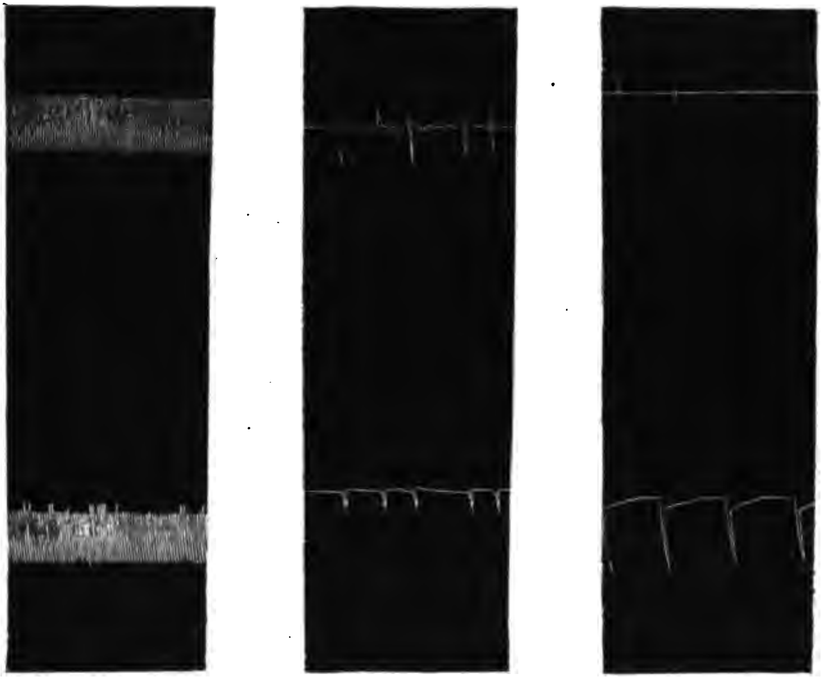

Fig. 3

Part 1. Normal respiration.
Part 2. Respiration after section behind the posterior corpora quadrigemina
Part 3. Respiration after section of the dorsal spinal roots in the cervica and upper thoracic regions. Upper tracing represents costal, lower abdomina respiration.

Section of the dorsal roots before the corpora quadrigemina are sectioned does leave some additional effect to be gained by the latter operation, as the following protocol shows.

March 5, 1918. Male cat (fig. 4).

Ether, tracheotomy, laminectomy.

2.45 p.m. Control tracing (part 1).

3.05 p.m. After section of the dorsal roots in the thoracic and lower cervical regions (part 2).

3.30 p.m. After section of the posterior corpora quadrigemina. Note the Cheyne-Stokes respiration (part 3).

From this experiment it is evident that there are still some afferent intercostal impulses going through until the posterior corpora are divided—not until then are all intercostal impulses cut off.

Such corroborative detail points strongly to the probability of the existence at the level of the posterior corpora quadrigemina of some station closely related to the integration of the afferent impulses from the respiratory "cage."

While certain of the motor impulses concerned in the skilled movements of respiration must originate in the motor areas of the cerebrum it is hardly likely that these are called into play during normal respiration or during anaesthesia; and on the other hand, a purely medullary type of respiration due to the movements of the diaphragm alone is not normal either. I believe, therefore, that sensory fibers from the dorsal roots of the spinal nerves from the intercostals travel up the brain stem as high as the level of the posterior corpora quadrigemina, where some connection with the descending motor fibers is effected. In other words, the dorsal spinal nerves and a region for the integration of respiratory impulses at the level of the posterior corpora belong to the same system. The fact that vagi and mesencephalon are unrelated in this manner is what makes section of the vagi in this connection so much more fatal than section of the dorsal roots—a relation which will be discussed in a subsequent paper.

The time element concerned in section of the dorsal roots and the corpora quadrigemina may also be considered. It is well known from clinical evidence that people in whom accident or disease has destroyed the dorsal spinal nerve roots are able to support life very adequately. Stewart (12) has cited the case of a man in whom all the ribs became completely immovable from disease of the spine in the lower cervical region. He was able to lead an active life and carry on his business although he breathed entirely by means of the diaphragm and abdominal muscles. Whether an animal in which both the dorsal roots and the corpora quadrigemina were destroyed could maintain life very long, I am not prepared to state; but experimentally, under anaesthesia, good respiration may be maintained for several hours subsequent to these operations.

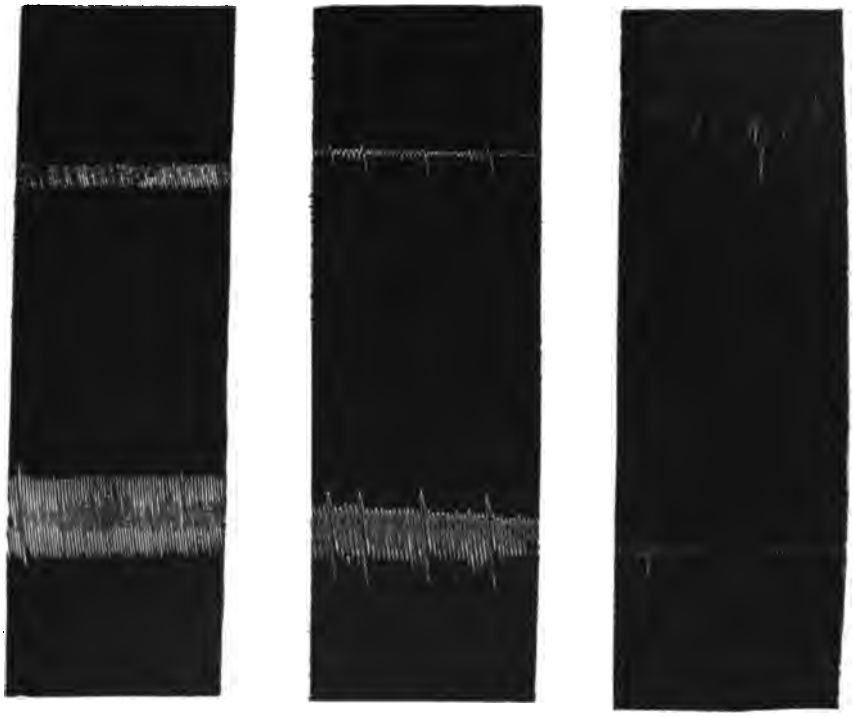

Fig. 4

Part 1. Respiration after laminectomy.

Part 2. Respiration after section of the dorsal roots in the thoracic and lower cervical regions.

Part 3. Respiration after section behind the posterior corpora quadrigemina. Note the Cheyne-Stokes respiration. Upper tracing represents costal, lower abdominal respiration.

Sherrington (13), in his work on decerebrate rigidity, describes the persistent tonic spasm which occurs in certain groups of muscles after section of the brain stem in front of the corpora quadrigemina. The groups of muscles which are contracted are the retractors of the head and neck, the muscles of the tail, the extensors of the elbow, knee, shoulder and ankle—the antigravity muscles. The spasm depends on the integrity of the dorsal spinal roots and appears not at all, or only imperfectly, in the limbs of which the corresponding dorsal nerve roots are divided. Section at that level of the corpora quadrigemina also does away with decerebrate rigidity, a fact which offers further confirmation that certain fibers of the dorsal spinal nerve roots have end stations at this level.

CONCLUSIONS

In summarizing the effects upon the respiratory movements of section of the dorsal roots of the spinal nerves and at the level of the posterior corpora quadrigemina, my findings are:

1. Section of the dorsal roots of the thoracic and cervical spinal nerves results in a diminution or cessation of active costal respiration. The effect of section of both thoracic and cervical nerves is a more marked diminution of costal respiration than after section of the thoracic roots alone. After section of the thoracic roots, abdominal respiration remains unchanged and there is no marked alteration in the respiratory rate.
2. Section of the brain stem below the anterior corpora quadrigemina results in a slower, deeper form of respiration than normal somewhat similar to the most severe effects which follow double vagotomy. Abdominal respiration is more prominent than costal.
3. Section of the dorsal roots of the spinal nerves after section into or behind the posterior corpora quadrigemina produces no more severe effect than section of the posterior corpora quadrigemina alone.
4. Section of the posterior corpora quadrigemina subsequent to section of the dorsal roots of the spinal nerves produces an effect on respiration somewhat greater than when the dorsal roots alone are sectioned.
5. The general relationship of afferent to efferent spinal nerve roots which Sherrington (14) describes obtains also in the afferent and efferent intercostal roots.

I wish to express my thanks to Professor F. H. Pike of this department for his valuable suggestions and criticisms of this work.

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VII. THE EFFECT OF ADRENALIN ON THE IRRITABILITY AND CONTRACTILITY OF MAMMALIAN NERVE-MUSCLE PREPARATIONS AFTER DEATH

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That the reaction of a muscle after fatigue is acid to litmus has been known for over half a century. Du Bois Reymond (1) in 1859 demonstrated that muscles become acid upon stimulation. Professor Schwann wrote in a letter to Du Bois Reymond of chasing a chicken until it was completely exhausted, after which he killed it and found a distinct acid reaction in the muscles. During the same year Funke (2) made note of this reaction. Four years later Ranke (3) was able to demonstrate that this acid reaction was caused by a production of lactic acid, and in 1865 he proved definitely that paralactic acid, monopotassiumphosphate and carbon dioxide are the products given off during the process of fatigue and that if these substances are injected into the circulation or irrigated through the muscles of unfatigued animals, typical fatigue results are obtained. Heidenhain (4), Landau and Paculy (5), Marcuse (6), Gleiss (7), Landsberger (8), Boehm (9), Osborne (10), Fletcher and Hopkins (11) and Fletcher (12) have confirmed Ranke's results. Lee (13), Burridge (14) and Schenck (15) were able to reproduce the fatigue curve by irrigating muscles with the above products. Geppert and Zuntz (16) have shown that the alkalinity of the blood is diminished after muscular exercise.

A similar acid reaction is always found in a muscle approaching a state of rigor mortis. Here as in fatigued muscles the reaction is the result of the accumulation of lactic acid (Du Bois Reymond (1), Ranke (3), Boehm (9), Osborne (10), Fletcher and Hopkins (11) and Fletcher (12)). Fletcher and Hopkins found that in frogs most of the lactic acid is produced while the muscle is still flaccid and irritable, before rigor has been reached. Fletcher noticed that in mammals the accumulation of lactic acid takes place most rapidly during the first three hours after death (0.453 per cent at the end of three hours and 0.513 per cent at the end of nine hours, extracted in the form of zinc lactate).

This series of experiments was made in order to determine, since the chemical and physiological changes in the two conditions are the same, whether adrenalin, which has a marked bettering effect on fatigued muscles (17), might not also affect muscles approaching rigor.

METHOD

The operative procedure and the apparatus used here were the same as those employed by one of us in a previous work (17). Cats were used. The tibialis anticus muscles were prepared for perfusion and at the moment that the cannulas were inserted into the vessels the animals were killed with ether. The nerve was exposed and placed in a Sherrington shielded electrode. The tendon of the muscle was fastened to a muscle lever and stimulated ninety times per minute. The medium for irrigation was a warm (37.5°C.) Ringer's solution, at a pressure of 70 cm. of water, containing only the oxygen absorbed from the air.

RESULTS

It was observed in these experiments that muscles lost their irritability to electrical stimuli with varying rapidity. Some muscles showed clearly at the end of an hour the effect of diminished supply of oxygen by the decreased irritability to the electrical stimulus and a decreased height of contraction. Others continued to respond almost normally at the end of that time.

Figure 1 was taken from an animal which had been dead for 1 hour and 6 minutes. Perfusion was begun 17 minutes before the beginning of stimulation. The muscle was able to do only one-third as much work as the corresponding muscle of the other limb had done, under similar operative conditions, immediately after the animal had been killed. The muscle in figure 1 contracted for 2.6 minutes, the contractions reaching at the highest point 2.7 cm., after which they dropped gradually to 0.5 cm., at which point, *1*, adrenalin (1 cc. of 1:100,000 solution) was injected into the perfusion fluid near the arterial cannula. This prolonged muscular contraction for 1 minute. At *2*, muscular contraction having almost ceased, adrenalin (0.5 cc. of a 1:1000 solution) was injected. Immediately there was produced a marked vasoconstriction and simultaneously an increase in the height of muscular contraction, at its highest point 29 fold. The effect of this injection lasted more than 7 minutes. This increase in height of muscular contraction is 11 per cent higher than the first response of the muscle recorded here. Adrenalin therefore not only counteracted the effect produced by fatigue but also a part of the decreased efficiency of the muscle caused by the lack of blood supply and the consequent death changes.

During this research it was repeatedly noticed that the threshold stimulus markedly increases during death changes. In some animals the muscle failed to respond to electrical stimulation of its nerve after the circulation had been removed for one hour. In others the threshold stimulus was increased five to one hundred times above normal. In all cases where any response could be obtained adrenalin lowered the threshold stimulus. An example may be cited here. In one animal in which the circulation was removed for 1 hour and 47 minutes, the current strength necessary to arouse the muscle into activity upon electrical excitation through its nerve was 28 Z units (18). In the opposite limb the corresponding muscle at death had responded to a

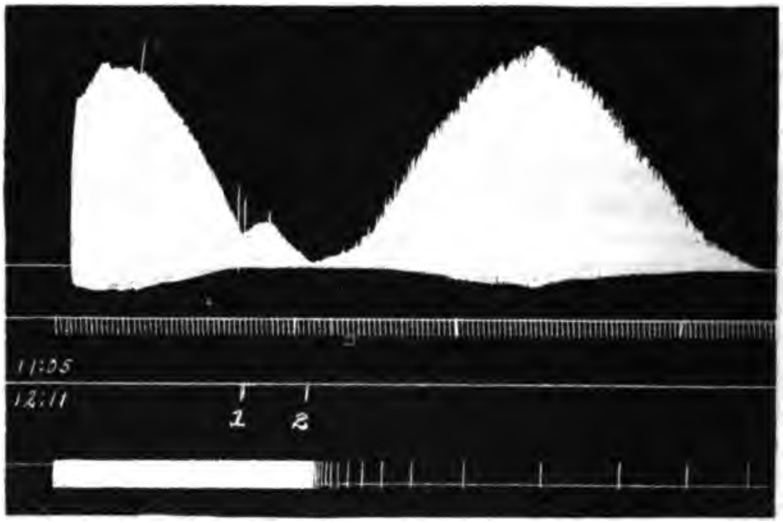

Fig. 1. In this and the following figures the upper curve is the record of muscular contractions, below it the time interval. The lowest record indicates the rate of flow of the perfusion fluid through the muscle. *1*, adrenalin 1 cc. (1:100,000); *2*, adrenalin 0.5 cc. (1: 1000). Time in 5 seconds.

stimulus of 2 Z units. After an injection of adrenalin (1 cc. of a 1:100,000 solution) the threshold of 28 Z units was decreased to 10 Z units. Another injection of adrenalin (0.5 cc. of a 1: 1000 solution) lowered it to 6 Z units. This demonstrates that the action of adrenalin in muscles undergoing death changes is similar to that observed in fatigued muscles.

Figure 2 is a record obtained from a kitten weighing 1.5 kilos which received an intravenous injection of 15 mgm. of hirudin as an anticoagulant. The animal was killed at 2:32 and the perfusion was begun at 3:08 (36 minutes later). At 3:15 or 42 minutes after the animal was killed and 6 minutes after perfusion was started stimulation of the muscle at the rate of ninety times per minute was begun. The muscle responded only slightly to a strength of stimulus of 485 Z units. (See *x*) At *1* and *2* injections of adrenalin chloride (2 cc. of 1:100,000 solution) were made into the perfusion fluid. There was, as a result of the first injection, approximately a 14 fold increase in the height of muscular contraction and as a result of the two injections the betterment in muscular contraction lasted for more than 5 minutes.

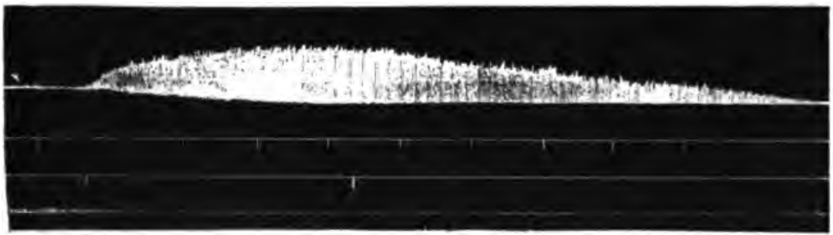

Fig. 2. Adrenalin 2 cc. (1: 100,000) injected into the perfusion fluid at *1* and *2*. Both make and break shock contractions present. Time in 30 second intervals. Reduced to $\frac{3}{5}$ original size.

Figure 3 is presented to show the effect of adrenalin on nerve-muscles in which the irritability was completely lost even to strong electrical currents. The animal from which this record was made had been dead for 1 hour and 5 minutes. In the beginning of the experiment the strength of the current was not known but the pointer of the secondary coil stood at 0, indicating a current of more than 799 Z units. The muscle was perfused for 12 minutes before excitation was begun. At *1*, indicated in the record, adrenalin (2 cc. of a 1: 100,000 solution) was injected in the usual manner and in less than 1 minute the muscle responded vigorously to both make and break shocks. To eliminate the make shock contraction, the current was decreased to 220 Z units at *12* but due to the adrenalin the irritability of the nerve-muscle gradually increased so that the current had to be decreased to 59 Z units at *15* before there was a permanent loss of the make shock contraction. The height of contraction increased from 0 to 1.8 cm. as a result of the injection. At *2* another 2 cc. of adrenalin (1:100,000) was injected which brought about a betterment of more than 60 per cent. In the corresponding muscle of the opposite limb, from which the circulation had been cut off for 2 hours, three injections of 2 cc. each of adrenalin (1: 100,000) were necessary before the muscle could be made to contract.

This recovery of contractility and irritability after total loss could not be brought about by adrenalin in every case. It was observed that the muscles of animals dead for three or four hours could not be restored to activity by adrenalin upon excitation of their nerves. It was noted, however, that in some of these cases adrenalin was capable of restoring the muscle fibers to activity by direct excitation of the muscle, individual fibers responding by twitching.

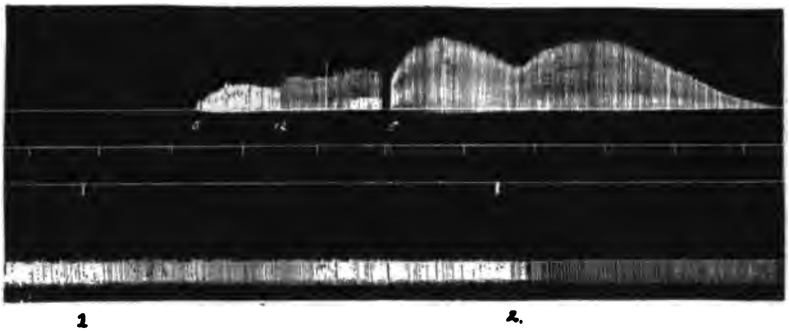

Fig. 3. Explained in text. *1* and *2*, adrenalin 2 cc. (1: 100,000). Time in 30 seconds. Reduced to ⅔ original size.

DISCUSSION

Adrenalin affects muscles undergoing death changes as it does fatigued muscles. It increases muscular activity (the height of muscular contraction) and increases the irritability of the nerve-muscle to electrical excitation (decreasing the threshold stimulus).

It probably acts upon the same substance in these muscles as in fatigued muscles. Here as in fatigued muscles there are three possible points of action:

a. It may assist or hasten the conversion of glycogen into available sugar to be used as energy. Since sugar is used in muscular contraction it is conceivable that any change which would hasten its production would better the height of muscular contraction. In muscles undergoing death changes much of the glycogen has been changed into lactic acid. A considerable quantity, however, probably remains from one to three hours after death to be converted into sugar, which conversion may be assisted by adrenalin.

b. Adrenalin may hasten the reconversion of lactic acid into sugar so that more available energy is present. (Transformation of fatigue products.)

c. The oxidation of lactic acid into carbon dioxide and water may take place more rapidly with the aid of adrenalin, a toxic substance thus being changed into less harmful substances. (Destruction of fatigue products.)

SUMMARY

Adrenalin has the same action upon nerve-muscle preparations undergoing death changes as it has upon fatigued muscles. It decreases the increased threshold stimulus and betters the height of muscular contraction.

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A NOTE ON THE SUPPOSED RELATION OF THE SYMPATHETIC NERVES TO DECEREBRATE RIGIDITY, MUSCLE TONE AND TENDON REFLEXES

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The question of the sympathetic innervation of striated muscle is still far from settled. As the evidence accumulates, the probability diminishes that a simple explanation of tonus has at last been found. For a time the researches of Boeke in anatomy, and of the physiologist here quoted, had made it seem probable that tonic muscular contraction was due to sympathetic innervation. In 1913 Dusser de Barenne (1) tried the effect of unilateral section of the abdominal sympathetic chain in decerebrate cats, and found that in only five out of nine such preparations was there an ipsilateral diminution of the rigidity. He concluded that the tonic impulses causing decerebrate rigidity did not reach the muscles of the hind leg by way of the sympathetic. The next year Langelaan (2), writing on tonus, stated that decerebrate rigidity was to be regarded as a spasm of sympathetic origin. In 1915 deBoer (3) published a monograph on tonic innervation, summarizing his work of several years, and gave evidence to show that extirpating the abdominal sympathetic chain on one side in cats caused ipsilateral hypotonicity with exaggeration of the tendon reflexes. The loss of tone showed in the affected hind leg by its diminished resistance to passive flexion and by the fact that when the cat was held up by the skin of its neck, this limb hung lower than the contralateral one, and showed greater extension at the joints. The animal's tail was observed to hang toward the unaffected side.

In 1916 these experiments of deBoer and those of Dusser de Barenne were modified and repeated and the results of the work on mammals are here published. A series of experiments was also done on frogs to test deBoer's observation that cutting the rami communicantes of the abdominal sympathetic in frogs causes a loss of tone in the ipsilateral leg muscles. Sixty-one frogs were operated on in different ways and although the simple cutting of the rami usually seemed to cause the leg to hang lower, no consistently corroborative evidence was obtained from stimulation or degeneration experiments. These results are considered too equivocal to be worth publishing at length.

More recently Van Rijnberk (4) has repeated the experiments of Dusser de Barenne and finds that in no cases does the section of the abdominal sympathetic affect the development of the decerebrate rigidity. Dusser de Barenne (5), however, has repeated deBoer's experiment and has found a lessening of muscular tone in the corresponding hind leg after cutting the abdominal sympathetic chain.

In my mammalian experiments the abdominal sympathetic was cut before decerebration in five cases and afterwards in one case. The effect of stimulating the sympathetic chain was tried out, also the effect of inhibiting decerebrate rigidity by cerebellar stimulation[1] with and without an intact sympathetic chain. Besides this, repeated observations were made on six cats, after their recovery from the sympathetic excision, to see if the muscular tonicity or tendon reflexes had been affected.

CONDENSED PROTOCOLS

Cat 1. February 9, 1916. Operated under ether and aseptic precautions; flank incision and extraperitoneal excision of 4th and 5th lumbar ganglia on the right.

February 10. No evidence of hypotonia of right leg or tail.

February 11. No evidence of hypotonia of right leg or tail.

Abdominal operation, with excision of 6th and 7th lumbar ganglia and 1st sacral ganglion, on the right.

February 15. No evidence of hypotonia of right hind leg; it is somewhat more stiffly held than the left. Tail carried evenly. Operation for decerebration: transection just anterior to superior colliculi; strong rigidity immediately, equal on the two sides. Four hours later rigidity still present and equal bilaterally. Cat killed. Autopsy substantiates operation.

Cat. 2. February 19, 1916. Extraperitoneal excision of 6th left lumbar sympathetic ganglion.

February 20. No evidence of hypotonia.

---

[1] In a previous paper (6) the writer has described how stimulation of the anterior lobe of the cerebellum on either side, or direct stimulation of the underlying superior cerebellar peduncle, causes ipsilateral inhibition of the extensor rigidity in decerebrate cats.

February 21. Tail hangs to right more than to left, but not definitely. Legs show no difference. Decerebrated: immediate rigidity, equal on the two sides, tail stiff and in midline; observed for one hour. Autopsy shows 6th left lumbar ganglion absent.

Cat 3. February 23, 1916. Operation; piece of abdominal sympathetic 1.5 cm. long excised with 5th right lumbar ganglion.

February 24 to March 17. Cat kept alive and observed for twenty-three days; tail always hung in midline. With 50 gram weight attached to each hind leg and the cat held up by the neck, the legs hang at varying heights; on four days the right seemed definitely lower, on two days the left, and on four days there was no difference. No increase in knee jerks on either side; no loss of resistance to passive motion found in either hind leg.

March 17. Decerebration: rigidity develops after five minutes, more on right than left, later equal on two sides, tail stiff in midline. Stimulation of anterior lobe of cerebellum to right and left of midline gives ipsilateral inhibition of the extensor rigidity. Autopsy shows 5th right lumbar ganglion of sympathetic chain to be lacking; the nerve trunk is divided and embedded in scar tissue.

Cat 4. February 29, 1916. Extraperitoneal operation; left abdominal sympathetic chain cut between the 4th and 5th lumbar ganglia.

March 1. With 50 gram weights attached to legs, the left usually hangs lower than the right.

March 4, 9 and 10. Tail hangs in midline, no difference in tonus of the legs either on passive motion or on holding animal up with weights on legs. No difference in knee jerks.

March 10. Decerebration: slight rigidity for three hours, equal on the two sides. Autopsy checks up operation.

Cat 5. March 6, 1916. (Decerebration before cutting sympathetic.) 11.45: Decerebration: rigidity comes on quickly, equal on two sides.

12.30 Exposure of right sympathetic chain, loose ligature laid around it.

1.50: Right Achilles tendon attached to lever recording on drum. Rigidity medium throughout this time.

2.20 to 3.30: Tetanic induction shocks to the sympathetic nerve at the ligature (between 4th and 5th lumbar ganglia) cause no increase in the tonus of the right gastrocnemius recording on the drum.

3.40: During good rigidity, the sympathetic is cut at the ligature and no relaxation of the right gastrocnemius occurs. After this, rigidity remains equal on both sides in the legs and tail.

4.30: Cerebellum exposed and electrical stimulation of the anterior lobe in the midline causes inhibition of the rigidity, as is shown by relaxation of the right gastrocnemius.

Cat 6. March 10, 1916. Abdominal operation; sympathetic chain exposed and the 6th and 7th right lumbar ganglia avulsed with a portion of the nerve.

March 13. Tail hangs slightly to left; knee jerks greater on left; legs hang equally when weighted.

March 15. Tail hangs slightly to right; knee jerks constantly greater on right; less resistance to passive motion on right; left leg hangs lower with 50 gram weights.

March 16. Decerebrated: rigidity comes on in five minutes; moderate intensity, equal bilaterally. Each Achilles tendon attached to a lever registering on drum. Cerebellum exposed and effect of stimuli registered; preparation continues active with rigidity for four hours. Tetanic induction shocks to anterior lobe of cerebellum cause inhibition of rigidity in both hind legs, as in cats with intact sympathetic nerves (6). Autopsy checks up operation; bronchopneumonia present.

Cat 7. March 14, 1916. Abdominal operation; dissected out 3d, 4th and 5th right lumbar ganglia with intervening sympathetic nerve.

March 15. Tail hangs slightly to left; knee jerks greater on left; less resistance to passive motion on right; with 50 gram weights left leg hangs lower than right.

March 16. Animal died; peritonitis; autopsy checks up operation.

SUMMARY

Examination of the above protocols shows that in five cats in which unilateral division of the abdominal sympathetic chain had been previously performed (three to twenty-three days before decerebration) the development of decerebrate rigidity in the hind legs was unaffected by this operation. In three cases the anterior lobe of the cerebellum was exposed and stimulation with induction shocks was tried. In all three inhibition of the rigidity was recorded as in normal animals (6).

In six cats observations were repeatedly made of the knee jerks, the tonus of the hind legs and the tonus of the tail. Previous investigators have described hypotonia of the leg on the side of the operation; this lack of tone was said to have appeared when the animal was lifted by the neck—the more flaccid leg hanging with its foot lower than that of the more tonic, equal weights being attached to the legs to bring out the phenomenon. No such ipsilateral hypotonia was found in these experiments nor was there any constant change in the knee jerks. Passive flexion of the hind legs was tried repeatedly and no constant difference could be detected between the operated and unoperated sides. It was noticed, however, that slight differences in grasping and holding the cat's neck caused changes in the hanging of the legs and in their stiffness; there seemed to be a synergic relation between the position of the neck and the tonus of the hind legs (7), which might explain some of the former observations.

Hypotonia of the tail, as described by deBoer, shows by an asymmetrical posture of the tail. It was described as being held toward the side on which the sympathetic was intact, the theory being that the muscles of this side of the tail had the greater tonus. In six of the cats of the present series the position of the tail after operation was noted. In two it seemed to hang slightly toward the intact side, suggesting an ipsilateral hypotonia; in one it consistently deviated toward the operated side and in three there was no deviation from the normal midline position.

In one case the decerebration operation was performed first and later the sympathetic was exposed, stimulated and finally cut while the tonically contracted gastrocnemius was registering on a revolving drum. No change in the steady contraction was recorded.

CONCLUSIONS

Section of the abdominal sympathetic chain in cats:

1. Has no effect on decerebrate rigidity, either by preventing its development or its inhibition.
2. Causes no obvious hypotonicity of the hind legs or tail.
3. Causes no change in the tendon reflexes.

Stimulation of the abdominal sympathetic chain causes no tonic contraction of the ipsilateral hind leg.

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THE NON-EFFECT OF CORPUS LUTEUM PREPARATIONS ON THE OVULATION CYCLE OF THE RAT

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Received for publication May 16, 1918

The first and one of the most attractive of the many theories as to the function of the corpus luteum of the mammalian ovary is that suggested by Beard (1) and reiterated by Prenant (2), that this gland may by an internal secretion inhibit ovulation during pregnancy in order to spare the organism for a time the stresses of the ovarian cycle which, as Beard supposed, by finally asserting themselves bring about parturition; if the ovulation cycle reappears prematurely, abortion occurs and hence the necessity of some such inhibitory mechanism as the corpus luteum was supposed to furnish.

Though Beard's theory as a whole did not retain general interest, that part of it which refers to the corpus luteum rests upon the undoubted fact that there is a pause in ovulation between the regular periods and during pregnancy, and hence the possibility of an inhibitory function of the corpus luteum is not to be neglected.

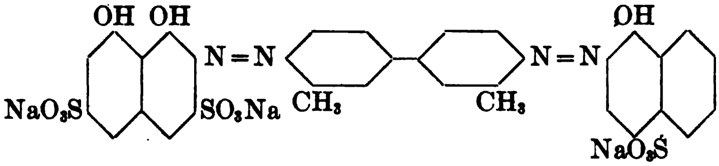
Among the mass of speculative discussions of this subject there have been two important efforts to test the hypothesis by experiment. Leo Loeb (3) attempted to determine the normal period of ovulation in guinea pigs and then to remove the corpora lutea by operation and to determine the time of the ensuing ovulation. As a result of his experiments he believed the period between two ovulations to be variable but usually from twenty to twenty-five days, and never less than fifteen days. If the corpora lutea are removed, the total interval between the ovulation previous to operation and that next following is from twelve to sixteen days, usually about fourteen. The occurrence of ovulation was determined by killing the animals at varying times of the cycle and studying the ovaries. Recently Stockard and Papanicolaou (4) have introduced a much more exact method of following the reproductive cycle in living animals by the observation of vaginal changes, and have determined the ovulation cycle of the guinea pig to vary from fifteen to seventeen days, averaging fifteen and one-half. Thus it would seem that Loeb's experiments do not prove an acceleration of ovulation following the removal of the corpora lutea.

Pearl and Surface (5) have adopted the ingenious method of administering corpus luteum substance to hens, in which the function of ovulation is more frequent and much more easily measured than in mammals. In a small series of carefully guarded experiments, using hens which were laying regularly, the intra-abdominal injection of the commercial desiccated corpus luteum substance prepared by Armour & Company, in doses of 0.4 to 0.5 gram per kilogram of body weight, suspended in normal saline solution, produced in most cases an inhibition of ovulation lasting for several days, and in all cases the number of eggs laid in the ten days following the injection was markedly less than in the same period before treatment. The administration of normal saline alone and of boiled corpus luteum substance, did not produce inhibition of ovulation.

It has seemed to the present writers that an application of the same experiment to mammals would be worth an attempt, in spite of the great difficulties attending the determination of ovulation in the viviparous animals. We need first an animal in which ovulation is known to be frequent and fairly regular, and second, a sure means of detecting the occurrence of ovulation during the period of experiment. Our work was concluded before we could make use of Stockard's observations, and meanwhile we had developed an experimental method which gives an exact count of the number of follicles which have ruptured during a given period, by direct inspection of the ovaries and without the uncertainties formerly occurring. This method we owe to the generous advice of Professor Evans and Dr. J. A. Long of this University, who put freely at our disposal the results of their long studies upon vital staining of the corpus luteum and of the ovarian cycle of the adult rat, Mus norvegicus.

The studies of Drs. Long and Evans, now in course of preparation for publication, show that the ovulation cycle of the rat varies from four to eleven days but may be fairly constant for any one rat. Ovulation always occurs about eighteen hours after the birth of a litter, and we can make this a convenient starting-point for following the cycle by administering a vital stain which will color all corpora lutea existing in the ovary. (In this animal the corpora persist for many weeks, so that several successive crops are present at one time.) Follicles rupturing subsequently to the time of staining will give rise to unstained corpora lutea.

The dye furnished us by Doctor Evans was a salt-free sample of a benzidine compound of blue color having the following formula:



His tests had shown that this dye, when administered subcutaneeously or intra-abdominally, is readily taken up by the lutein cells and stored by them for long periods, so that the corpora lutea take on an intense blue color; that the dye is not unduly toxic in proper doses, and that with certain precautions it withstands the manipulations necessary to the preparation of paraffin sections. Furthermore, a long series of experiments by Evans and Long proves that the dye as used for vital staining of the ovaries does not of itself inhibit ovulation in rats.

The animals were from a stock resulting from the inter-breeding of albinos with a few rats of the ordinary brown variety. On the day of littering and three succeeding days they were given intra-abdominal injections of 4 cc. of a sterilized 1 per cent solution of the dye in 0.7 per cent NaCl. On the fifth day we began the administration of corpus luteum substance. Two preparations were used, one being Armour's desiccated corpus luteum substance from cows, extracted with petroleum benzene to remove the fats (the method of preparation is given by Fenger (6)); the other was that prepared by Hynson, Westcott & Dunning, which we are informed by the makers undergoes no changes in its preparation except such as are incidental to the careful desiccation. We are very much indebted to the firms mentioned for their courtesy in furnishing the materials for our work. In administering the corpus luteum substance all aseptic precautions were used except that the powder itself could not be sterilized. The powder was suspended in sterile normal saline solution by means of a mortar and pestle.

Ten rats, stained as described, were each given ten doses of the Armour preparation on alternative days, each dose consisting of 200 mgm. of corpus luteum substance in 3 to 4 cc. of normal saline. As the average body weight of the animals was 175 grams, the dose represented somewhat more than 1 gram per kilogram of body weight, and the total amount received by each rat was 4 grams, equivalent to about 16 grams of the fresh substance.

On the twenty-fifth day after littering the animals were killed, there having been time for two, three or four ovulation cycles to have elapsed, according to individual variations of the rats. All had gained a little in weight (they were young animals) and autopsy showed all but one to be in good condition, except for intra-abdominal adhesions due to the granular, partly insoluble and unsterile nature of the injected substance. The material had been well absorbed in all cases. One rat showed a severe recent peritonitis. The numbers of stained and unstained corpora lutea were noted and then carefully checked under the microscope in serial sections; to which end the ovaries were fixed by injection of Zenker's fluid through the aorta, removed after three hours and washed in 70 per cent alcohol, dehydrated, cleared with xylol and imbedded in paraffin. The serial sections were stained on the slide with alcoholic carmine. It is important to avoid aqueous reagents in order not to dissolve the microscopic dye-granules.

In all the animals, including the one which was suffering from peritonitis, ovulation had continued unchecked, as proven by the presence in their ovaries of new unstained corpora lutea numbering from thirteen to thirty. The individual counts of stained and unstained corpora are very similar to those found in stained animals which have not received corpus luteum substance, after the same number of days. In one of the experimental animals the normal ova of a recent ovulation were discovered in the Fallopian tubes.

One rat received the Hynson, Westcott & Dunning preparation, but in daily doses, thus receiving twice as much as was given the first ten animals. This dose was sufficient to cause great emaciation and many adhesions, but there were ten unstained and thirteen stained corpora lutea in the two ovaries. A control animal killed the same number of days after littering showed thirteen unstained and twenty-seven stained corpora lutea.

CONCLUSION

The intraperitoneal injection of large doses of desiccated mammalian corpus luteum substance does not inhibit ovulation in the rat.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. No. 91

ON THE SIGNIFICANCE OF THE REACTION TO SHADING IN CHITON

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I. The objections which certain writers have urged against the theory of phototropism in animals, as originally propounded by Loeb and so brilliantly developed and supported by his later quantitative researches, are of considerable variety: thus upon the one hand we note that in different cases the fact of photic orientation is either denied, or its method is stated to be indirect, or it is dismissed as a "laboratory product" (which is quite beside the point); while upon the other hand we find, for cases where the orientation-process itself is unmistakably clear, that exception is more specifically taken to the conception of direct photic excitation, which the tropism theory advances. It is with an exception of the latter category that we propose to deal in this paper.

Developing an idea originally made prominent by Jennings, Mast ('11, '14) has been the most active defender of the proposition that the orienting stimulus in photic responses can in general be traced to the "time rate of change of intensity" of the incident light upon photoreceptors. This view of the method of excitation in phototropic movements obviously demands, among other things, at least these two preliminary conditions: (1) A change of the incident light intensity must actually be shown to take place with an effective "time rate;" and (2) the known response to change in light intensity, when such form of reaction takes place, must be of a character appropriate to produce orientation of the kind (negative or positive) actually exhibited by the particular animal concerned. The latter condition is, as a matter of fact, very generally realized; thus Mast remarks ('11, p. 265):

> In many of the lower forms [how low is not indicated] orientation results from responses to change in light intensity. When these forms are negative they respond only to an increase of intensity, and when they are positive only to a decrease.

The behavior of some very slowly moving animals in an illuminated field gives valuable information relative to the first of the necessary conditions to which we have referred, and there are a few animals in which the second of these conditions can be subjected to examination. We are concerned in this communication with the photic behavior of an animal which seems especially valuable in relation to both of these conditions, but more particularly to the latter, since its response to change in light intensity ("differential sensitivity") is always of a character consistent with the sense in which photic orientation takes place. This animal is Chiton tuberculatus Linn., the common intertidal chiton of the Bermudas. The behavior of this 'primitive' mollusk has not previously received attention, presumably because of the reputation for persistent sluggishness which the Placophora in general enjoy; nevertheless, their activities prove, upon careful scrutiny, to exhibit a number of exceptionally interesting features.[1]

II. Young individuals of this species of chiton are almost always located under loosely piled, flat stones, in relatively dark surroundings, at about the upper limit of the tides. This is particularly true of specimens less than 2 cm. long; when still younger, less than 1 cm. long, the chitons are commonly found beneath low-water level. Somewhat older animals are also found in this type of habitat but after they attain a length of 6 cm. they commonly frequent more exposed situations. Chitons of the largest size (8 to 9 cm.) are most usually found freely exposed upon the intertidal zone of the shore rocks although they also inhabit crevices, semiconcealed depressions, the under surface of large flat rocks and other more or less dark places. Characteristically, however, the young chitons are found in dark situations, while the oldest ones live in the light.[2] When the photic responses of the young chitons and of the old, taken from these respectively characteristic habitats, are studied in comparison, it is found that the smallest chitons are uniformly photonegative, the larger ones photopositive. A miscellaneous collection of individuals may be caused to separate into two general size-groups by means of their respective reactions to sunlight falling obliquely upon them. They become oriented in a diagrammatic manner. It is necessary to study their reactions when immersed in seawater, for although the smallest specimens (less than 2 cm. long) move about actively upon a moist surface, the older ones do not creep freely when in air.

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[1] For an account of the behavior of this animal and an analysis of its reactions, we may refer to a forthcoming report by Arey and Crozier on "The sensory responses of Chiton" (cf. Arey, '18).

This difference in the behavior of the chitons of large and of small size respectively is clean-cut and thoroughly consistent when the extremes of size are compared. When animals of intermediate size, 5 to 7 cm. long, were studied it became clear that the sense in which orientation occurred depended upon the intensity of the light employed. An experiment illustrating this behavior may be cited: Owing to the relatively large size of the animals and to the slowness with which they move, it has been convenient, as well as necessary, to give careful attention to the responses of isolated single individuals.

Chitons 123.5, 123.7. Obtained from a deep crevice in the shore rock on the north side of Long Island. Placed in a dark container and protected from light until tested.

9.00 a.m. Placed in sea water in a shallow, rectangular glass vessel illuminated by diffuse light from a north window; each animal so placed that its long axis was transverse to the light.

9.07 a.m. Both chitons oriented *toward* the window, *toward* which each crept for 12 cm. in a straight line.

9.15 a.m. Animals placed as before at the center of the glass vessel, one headed into the light, the other transverse to it. Direct sunlight reflected horizontally upon the chitons.

9.16 a.m. Both began orienting *away* from the light; crept *away* from the light as far as possible.

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[2] The smaller chitons, consistently found under stones, are all less than two years old. With increasing age there is an increasing tendency to frequent illuminated areas; this correlation is not mathematically exact but is clear and unmistakable, so that individuals eight years old or older are almost always found in the light. The evidence supporting these statements regarding age cannot be given here, but will be found in a subsequent paper by one of the present writers (W. J. C.) treating of the bionomics of chiton.

9.25 a.m. Tested again, this time with light reflected from the north sky. Both animals diagrammatically positive.

10 a.m. to 12 m. Four further alternations of weak and strong light gave results completely consistent with the preceding.

Chiton 123.5 was a male 6.6 cm. long, no. 123.7 a female[3], 6.7 cm. long.

All that we wish to show at this point is that small (young) chitons are photonegative, the largest ones characteristically photopositive, and those of intermediate size positive or negative according to the intensity of illumination. The cause of this change in behavior is not important for our further conclusions in this paper, although, as we shall subsequently indicate, it has an important significance for the method of origin of some conspicuous bionomic correlations which this chiton displays.

III. Chiton tuberculatus is also reactive to shading. It is in fact remarkably sensitive to a very small, but sudden, decrease in light intensity. When a chiton is creeping quietly in moderately bright sunlight, the shadow produced by a fly, six feet distant, passing between it and the sun, will cause the animal's girdle to be firmly applied to the substratum; after a half minute or perhaps less the chiton continues its creeping toward the light, while in other instances small, momentary shadows produce merely a local depression of the girdle. *If the same individual chiton be caused to orient and to make locomotor progress away from a stronger source of light, it gives during this process reactions to shading which are of measurably greater amplitude.* Chitons of all ages and from every variety of habitat are consistent in their behavior toward shading. The reaction to suddenly decreased illumination is, to shadows of small area, local in character, and at its maximum consists (when the animal is creeping on a solid substratum) in ventral-ward contractions of the girdle, cessation of locomotion and contraction of the musculature of the foot and body generally; the magnitude of these movements depends largely upon the original intensity of the light and the amount of the chiton's surface which may be shaded. The response to shading is here essentially of that prompt, precise, local and predictable character which one finds exhibited in other mollusks and in various echinoderms and has led to its being loosely and somewhat gratuitously denominated a "reflex" to shading.

The ventral surface of chiton is also sensitive to shading. In this general distribution of differential sensitivity over its surface, chiton differs considerably from such photopositive gastropods as Conus and certain nudibranchs, for example. The response to shading of the ventral surface comprises, in cases where the response is maximal, a complete rolling up of the shell and contractive movements upon the head, foot, girdle and ctenidia. Under certain conditions this response on the part of Chitons fully extended but resting on their dorsal surface, is exceedingly delicate, both to shading, to touch and to other forms of excitation. Never under any circumstances does chiton respond to an increase in illumination, however, whether the dorsal or the ventral aspect of the animal is involved in the experiment, except as noted in the following:

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[3] Cf. Crozier ('18).

In older chitons the periphery of the girdle is sensitive, and reactive, to increase of light intensity, provided the final intensity be that of brilliant sunlight. The response is that already described, a depression of the girdle to the substratum. It is much less vigorous, much less complete, and more slowly carried out, than is a response to an equivalent shading. So far as it goes, this response completes the cycle of qualitative proof that changes of light intensity, as such, are of no concern in phototropic movements—because their sense is inconsistent with that in which orientation takes place. The girdle reaction to light suddenly made brighter is present under conditions in which the chiton is photopositive. Moreover, the girdle is not involved in photoreception leading to orientation, since this process remains the same when the girdle has been removed (by amputation).

IV. Thus Chiton tuberculatus is in its younger stages photonegative, in its later years of life characteristically photopositive, toward ordinary sunlight. Individuals of all ages, independently of the type of habitat from which they may be taken and regardless of the sense in which they are found to be oriented by light of a given intensity, indicate by motor responses of a uniform character that they are negatively reactive to sudden diminution in light intensity. They do not react in this way to a sudden increase in light intensity. It may be emphasized here, as in the case of certain pedate holothurians (Crozier, '14, '15), that the simultaneous presence of *photonegative* orientation and a precise *negative* response to shading, without any response (of the part concerned in orienting reactions) to increased illumination, is thoroughly inconsistent with the idea that photonegative orientation is bought about by a stimulation induced through any change in light intensity, as such.

The behavior of chiton is especially significant, however, since the older individuals are to various degrees (and in many cases very strongly) photopositive, though still fully reactive to shading; and since in any given chiton of medium size photopositive or photonegative orientation may be brought about at will by appropriately controlling the acting luminous intensity, yet without impairing the constant sense of its "differential sensitivity." We are, then, entirely at liberty to believe, and indeed forced to expect, that in other animals also photopositive orientation may have no organic connection of any sort with such sensitivity to shading as they may exhibit.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 310

REACTIONS OF FROGS TO HEAT AND COLD

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INTRODUCTION

The purpose of these experiments was to determine the behavior of the leopard frog, Rana pipiens Schreber, in water of different temperatures.

That the body temperature of the frog and of other cold-blooded vertebrates is very little, if at all, above that of their surroundings seems to be a well attested fact (Milne Edwards, '68; Rogers and Lewis, '16). Knauthe ('91) and Müller-Erzbach ('91) have studied the effects of low temperatures on the frog dealing more, however, with its resistance to cold than with its behavior. Knauthe found that frogs could survive a twelve hours' exposure to a temperature ranging from −1° C. to −5° C., during which their body temperature sank from −0.2° C. to −0.8° C. Most of the frogs failed to recover when the body temperature was reduced to −0.9° C. Müller-Erzbach froze frogs in water and exposed them to a temperature of from −4° C. to −6° C. for several hours. They afterwards revived.

Maurel et Lagriffe ('00) decided that the frog cannot survive a temperature of −5° C. but may survive a temperature of 0° C. to −3° C. These investigators studied the effect on the frog of temperatures from −4° C. to 41° C., pointing out a certain parallelism between the effects of abnormal cold and abnormal heat.

Torelle ('03) described the behavior of the frog at various temperatures. She found that the positive phototactic response in a dry box was much accelerated by raising the temperature to 25° C. Between 25° C. and 30° C. the frog grew restless. Above 30° C. it no longer responded to light. At a temperature of about 8°C. the frog became sluggish and was negatively phototactic. When placed in a jar of water at this temperature, the frog swam down to the bottom where it crawled about. It did the same when either the upper or lower two-thirds of the jar were darkened. The frog appeared stereotropic in water at temperatures between 4° C. and 10° C., trying to crawl under stones or get between them and the jar.

Babák ('13) also studied the stimulation of frogs by changes of temperature. A frog from which the forebrain had been removed increased the rate of its pharyngeal respiratory movements when a warm thermaesthesiometer was held at about 1 mm. from its skin and decreased the rate when cold was similarly applied.

Judging from the results thus far cited it seems that the frog can withstand a temperature at least as low as −3° C. It swims down and is sluggish in water at temperatures below 10° C., and it is especially active at temperatures from 25° C. to 30° C.

The present study of temperature reactions was suggested by G. H. Parker and carried on under his direction. Two main points were considered:

1. The percentage of time that a frog will spend at the surface of water at different temperatures in trials of fifty minutes duration; and
2. The number of times that the frog will go from the surface to the bottom and vice versa during these trials.

METHODS

In making observations on the frogs I used the following apparatus. Two glass jars 32 cm. high by 24 cm. in diameter, filled with water to a depth of 22 cm. were placed side by side on a small table. Thick wads of paper were kept under the table legs to exclude any disturbing vibrations that might be transmitted through the floor. Each jar was surrounded by a screen that cut off all light except that which came from in front. The jars were illuminated by diffuse light from the laboratory windows. The observer sat in front of the jars at a distance of 4 or 5 meters and the utmost care was taken not to disturb the frogs during an experiment by movements or noises. The screening of the jars was so arranged that the movements of a frog in one jar could not be seen by a frog in the other jar.

In making the tests, the jars were partly filled with water at the desired temperature and the frogs put in. The main stock of frogs was kept in a tank in the basement of the laboratory where it was light, but those to be tested were generally kept in jars in the laboratory for a day or so before the tests were made.

Each experiment began with a preliminary twenty-five minutes during which the frog was allowed to become accustomed to the new situation and to recover from the disturbance of having been handled. After this period had passed, record of the frog's behavior for the next fifty minutes was made, the whole proceeding thus requiring one hour and a quarter. Usually two frogs were tested at once, one in each of the two jars previously mentioned.

A graphic record was kept of the behavior of each frog. The temperature of the water at the beginning and end of each experiment was read to 0.1° C. from a calibrated thermometer graduated to degrees. The temperature of the water during the period of an experiment, one hour and a quarter, varied at most 5° C., a common change being about 2°C. The average for the fifty-minute trial was estimated by adding to or subtracting from the final temperature one-third the difference between the initial and final temperatures.

Ten records of this sort, obtained from ten different frogs, were made for every 5° C. from 0° C. to 35° C. Thus, each record of the first set has an average trial temperature between 0° C. and 5° C.; while those of the second set have average temperatures between 5° C. and 10° C. The first records were made on February 21 and the last on May 12. This time of year is usually not the most favorable one for work on frogs as many of them die, but tests were made on only such animals as were in good condition.

In discussing the results of these experiments, the general behavior of the frogs at the various temperatures may be taken up first, after which the interpretation of their behavior will be considered.

Temperatures from 0°C. to 5°C. When first placed in the jar, a frog generally swam down to the bottom immediately. This was usual at all temperatures because the animal was disturbed by handling. In water at temperatures between 0° C. and 5° C., the frog then commonly tried to regain the surface by a series of hard kicks, becoming more feeble as the animal grew stiff. It then settled down to the bottom by gravity. This was quite distinct from swimming down. It remained at the bottom practically all the time during the preliminary twenty-five minutes and the fifty minutes of the real trial. It was very sluggish, occasionally crawling around the bottom, moving the hind legs alternately, or making a few kicks toward the surface. If it reached the top of the water it usually sank down again very soon, frequently into an erect position with hind feet resting on the botton. In sinking, it sometimes fell over on its back and then righted itself gradually.

When quiet, it was crouched or stretched out on the bottom of the jar or erect. The frog was often apparently benumbed when taken from the jar, and would lie quietly on its back in one's hand. Maurel et Lagriffe ('00) note loss of the sense of equilibrium between 6°C. and 7°C. When put in water at room temperature, the frog became active in a minute or so.

Among the frogs tested one marked exception to this general description was noted. This frog floated 68 per cent of the time in water at a temperature of 1.5°C. It went from the top to the bottom of the jar forty times during the trial, swimming down rather than sinking. This frog behaved so entirely differently from the others that its record was omitted in plotting the curves to be discussed later.

The average temperature of the ten trials was 2.8°C. The time spent at the surface varied from none (eight cases) to four per cent, with an average of 0.5 per cent. The number of times the frog went between surface and bottom during the fifty-minute trial varied from none (five cases) to twelve, with an average of 2.2 times.

Temperatures from 5°C. to 10°C. Frogs in water at these temperatures were distinctly more active than in water from 0°C. to 5°C., though they were still somewhat sluggish. They generally swam rather than sank down and spent most of the time crouched on the bottom or crawling about there.

The average temperature of the ten trials was 7.1°C., the propor ion of time at the surface varied from none (three cases) to fifteen per cent, with an average of 4.2 per cent. The number of times the frog went up or down varied from none (one case) to forty-six, averaging 18.3 times.

Temperatures from 10°C. to 15°C. At these temperatures the activity of the frogs was much greater than in the preceding sets of trials. The animals swam up and down very freely and did not sink. They frequently floated at the surface, seldom, however, for more than a minute at a time.

The average temperature in the ten trials was 11.9°C. The frogs were at the surface from 3 to 48 per cent of the time, averaging 16.4 per cent. They swam up or down from two to one hundred and thirty-seven times during the fifty minutes, with an average of 55.4 times.

Temperatures from 15°C. to 20°C. At these temperatures the frogs were slightly more active than in the preceding set of experiments and floated more. In two cases the animals floated quietly nearly the whole fifty minutes of the trial. When they floated thus, they often had their hind legs spread out on the surface of the water; when they came up for a short time the hind legs generally hung down.

The average temperature of the ten trials was 17.6°C. The time spent at the surface varied from 1 per cent to 100 per cent, with an average of 37.9 per cent. The number of times the frogs went up or down varied from one to two hundred and sixteen, averaging 64.2 times.

Temperatures from 20°C. to 25°C. At these temperatures the behavior of the frogs was decidedly variable, but they swam up and down less than at temperatures slightly higher or lower. Some frogs floated quietly most of the time; others stayed crouched or occasionally erect at the bottom; others swam up and down repeatedly. In one instance the frog sank, somewhat as in cold water, instead of swimming down, taking at least fifteen seconds to settle from the top to the bottom.

The average temperature was 22.3°C. The frogs spent from 1 per cent to all of the time at the surface, averaging 52.6 per cent, and went up or down from none to one hundred and four times, averaging 26.7 times.

Temperatures from 25°C. to 30°C. The results of these trials were similar to those between 20°C. and 25°C. except that the average activity of the frogs was considerably increased in this set. However, there were some cases of quiet floating. The frogs showed some effects of the slightly unusual heat. Their movements tended to be rapid and jerky. One active animal usually swam down but sometimes sank; another fell over on its back at one time. This may have been due to a slight loss of the sense of equilibrium, which Maurel et Lagriffe ('00) found to be conspicuously the case in warmer water (34°C. to 36°C.).

The average temperature was 27.3°C. The proportion of time at the surface varied from 13 to 100 per cent, averaging 52.4 per cent. The number of times the frogs went up or down varied from none to one hundred and thirty-one with an average of 75.9 times.

Temperatures from 30°C. to 35°C. At these temperatures the frogs more clearly showed disturbance from the heat. When first placed in the jar they swam to the bottom as usual, then after a minute or so began swimming up and down rapidly. Sometimes the frog would then sink down, remain quiet at the bottom, give a spasmodic kick up and sink again with body held motionless. One frog did this ten times in eleven minutes during the preliminary twenty-five minutes, but stopped sinking before the period of the final test. Two continued sinking during this period. This sinking was not restricted to high or low temperatures For it was noted once that a frog sank in water at room temperature. However the tendency seemed much more marked in water below 5°C. and above 30°C.

Though decidedly active during part of the preliminary twenty-five minutes, the frogs were relatively quiet during the trial period, floating a good deal and making occasional sudden dives and splashes even when apparently undisturbed. The difference in behavior between the preliminary and the trial periods might be accounted for either by the assumption that the frog had become accustomed to the heat or by the fact that the water had cooled off a little. The temperature usually fell about 4°C. during the one hour and a quarter. The experiments of this set had the largest range of temperature.

The average temperature was 31.9°C. The frogs floated from 42 per cent to all of the time, averaging 57 per cent. The number of times the frogs went up or down varied from none to ninety-five, with an average of 30.6 times.

Temperatures above 35°C. Frogs put in water at a temperature of about 38°C. swam around very vigorously at first, then in a couple of minutes became quiet. They were promptly removed from the jar, motionless, as when taken from water at a temperature below 5°C. Placed in cold water, they revived in a minute or two. One frog was put in water at a temperature of about 42.5°C. It swam about with extreme rapidity and when taken out within about a minute lay on its back apparently dead. After being in water at room temperature about an hour, it swam a little but did not fully recover. Next day it was dead. Temperatures over 35°C. are probably above the physiological limit of normal activity.

DISCUSSION

The preceding descriptions may be summarized by means of curves, figures 1 and 2. In these graphs each curve is drawn from seven points. Each point in curve *B* of both graphs is based on the average of ten records, while the points in *A* and *C* represent the extremes in these records. In both graphs the abscissas represent temperature in degrees centigrade and the ordinates either the percentages of time at the surface (fig. 1) or the number of excursions made by the frogs (fig. 2).

In the percentage of time spent at the upper surface of the water (fig. 1) the lowest point on each of the three curves is not significant in showing a reaction, for below 5°C. the frogs did not swim but merely sank because of their inaction and specific gravity. This was the regular occurrence unless the frogs' throat or lungs were inflated with air.

In swimming down frogs frequently blew out bubbles of air. The average curve, *B*, shows a smooth rise between about 7°C. and 22°C., indicating the frog's tendency to seek deep water at low temperatures. The upper part of this curve is hardly significant. It merely shows that in warmer water the frogs were at the top about half the time, a condition that might well be attributed to chance without reference to temperature.

The curves that represent the maximum, *A*, and the minimum time, *C*, spent at the top indicate by their distance from each other the range of variability of the different frogs. This variability is least at the lower end of the curve, where the frogs were largely controlled by gravity, and it then increases rapidly, being greatest between about 17°C. and 22°C., where the temperature might be considered neutral and not strongly stimulating, thus leaving the frog's behavior more dependent upon other factors. At still higher temperatures, variability is somewhat reduced. Averages determined for a range of 5°C. from ten records having results varying as widely as these do are of course likely to be farther from the true averages than in cases where the records obtained agree more closely.

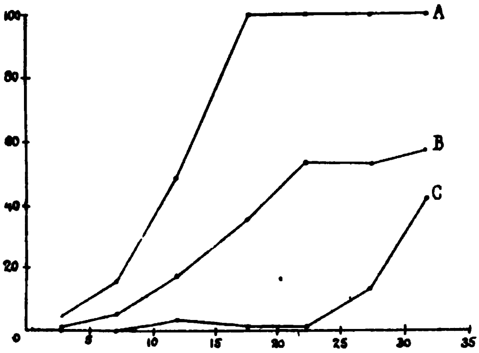

Fig. 1. The percentages of time spent by frogs at the upper surface of the water at temperatures varying between 0° and 35°C. The abscissas represent temperature in degrees centigrade. The ordinates represent in percentages the proportions of the 50-minute intervals spent by the frogs at the top of the water. Curve *A* shows maximum percentages, curve *B* average percentages and curve *C*, minimum percentages of time spent at the top. Each of the seven points in curve *B* represents the average of ten records, while the points in curves *A* and *C* show the extremes of these records.

The activity of the frogs as indicated by the number of times they went from the surface to the bottom of the water or vice versa, is shown in figure 2. This is a fair measure of their activity, for the jars were too small to allow much horizontal swimming. The curve for the average of the records, *B*, shows a marked increase in activity between about 3°C. and 12°C. It is doubtful whether the rest of the curve is of much significance, as the averages obtained could easily be far from true averages because of the great variation in the records. It is, however, of some interest to compare curve *B* with the observations of Maurel et

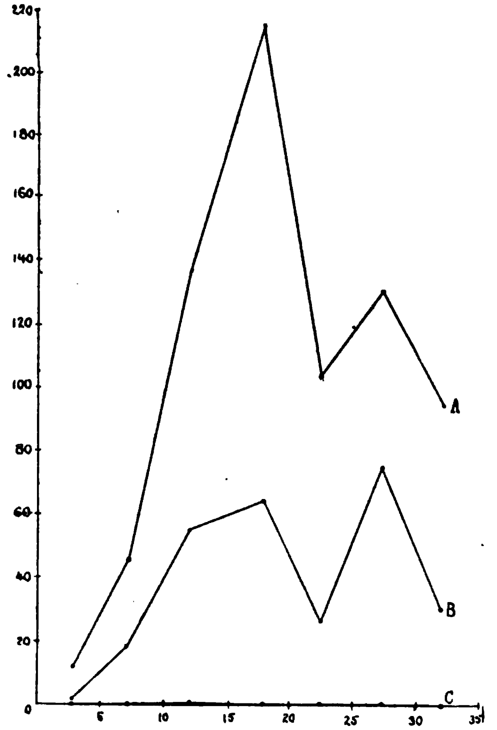

Fig. 2. The activity of frogs at temperatures varying from 0° to 35°C. The abscissas represent temperature in degrees centigrade. The ordinates represent the numbers of times the frogs ascended and descended in the water in each 50-minute interval. Curve *A*, maximum number of excursions; curve *B*, average number of excursions; curve *C*, minimum number of excursions. Each of the seven points in curve *B* represents an average of ten records, while the points in curves *A* and *C* show the extremes of these records.

Lagriffe ('00). They consider the interval from 20°C. to 25°C. to cover the normal temperature for the frog. This is a region of rather low activity on the curve. From 27°C. to 29°C. and from 11°C. to 15°C., Maurel et Lagriffe note "hyperexcitabilité." In curve *B* about 27.5°C. on one side of normal and about 17.5°C. on the other are points of greatest activity. It should be noted, however, that these are not sharply determined points but merely the averages of ten temperatures between 25°C. and 30°C., and 10°C. and 15°C., respectively. A decrease in activity above 27.5°C. and below 17.5°C. is apparent in the curve, corresponding to Maurel et Lagriffe's "hypo-excitabilité" between 30°C. and 33°C., and 8°C. and 10°C. Curves *A* and *C* together show the great variability of the records on which curve *B* is based and the consequent uncertainty of this curve for the higher temperatures.

SUMMARY

Below 5°C. the frogs became very sluggish and inactive. Gravity caused them to settle to the bottom.

Between about 5°C. and about 20°C. the colder it was the less the frogs were at the top of the water and the less active they were.

Between about 20°C. and 30°C. the frogs did not show any very definite reactions to temperature and their movements were highly variable.

Above 30°C. they showed somewhat decreased activity and a tendency to sink in the water.

Above 35°C. the heat had an injurious effect upon them.

Much more variation in behavior is shown at high than at low temperatures.

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ADRENALIN VASODILATOR MECHANISMS

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From the results of recent work regarding the vasomotor reaction to adrenalin several facts seem to have been established. It has been found that in anaesthetized cats and dogs the arterioles supplying skeletal muscle dilate when small quantities of adrenalin are injected into the circulation and that their reaction changes to constriction when the concentration of adrenalin is sufficiently increased (1), (2). The vessels of the intestinal tract have been found to give the opposite response since they constrict when small, and dilate when large doses are injected (3). It has been shown that there are many parts of the organism, bone (4), skin (5), spleen (3), (6), and possibly kidney (3), (7), the vessels of which show no active dilatation from doses of any strength. It is further conceded by the most recent workers (2), (8),(9) that all blood vessels which are dilated by small quantities of adrenalin lose this reaction, for the time being at least, when separated from central control by cutting their nerves. Dilatation under these circumstances is generally replaced by constriction. The reason for this change in reaction and the whole question of the mechanisms involved are still debated. It has been repeatedly suggested that the conflicting effects of adrenalin in varying concentration are entirely due to its stimulation of neuromuscular junctions of two kinds, one constricting and the other dilating. Those who hold this view believe that vessels which have been recently denervated fail to respond by dilatation to adrenalin because of loss of tone (9), (10).

Work from this laboratory has shown that stimulation of the sympathetic and dorsal root ganglia by adrenalin is sufficient to account for the dilatation (11). Although Gruber has shown that some time after denervation peripheral mechanisms respond in a similar manner, this might be due to loss of sensitivity by the constrictor myoneural junctions. The present research is an investigation of this problem.

METHODS

The methods employed are those of the previous researches described in this Journal, with some modifications and additions. Adrenalin chloride solution (Parke, Davis & Company) was used except in one experiment, in which a more concentrated solution was needed for direct application to ganglia. In this case we used pure adrenalin, made by the same firm. Blood pressure was taken from the carotid artery and injections into the general circulation were made by way of the jugular vein. In order to reduce the constrictor effects of the skin, we eliminated the paw by using a metal cuff open at both ends, a side-tube furnishing connection for the bellows. Both ends of the cuff were made air-tight by packing with a vaseline-cotton or vaseline-paraffin-cotton mixture. In the perfusion experiments, when records were to be taken of one hind limb only we put the cannula into the common iliac artery; when both limb volumes were being recorded we perfused through the abdominal aorta immediately above the bifurcation. The perfusion fluid was allowed to escape through slits in the iliac vein or veins directly into the abdominal cavity since any attempt to lead it away through cannulae from the veins resulted sooner or later in clotting. In some experiments we had difficulty in getting an equal flow of the perfusion solution to the two limbs. Results from these were of course discarded. The difficulty was found to be lessened by tying the internal iliac and the middle sacral arteries. The pressure employed for perfusion varied in different experiments between 10 mm. and 50 mm. Hg., the average being about 20 mm.

In all experiments involving denervation of a limb both the sciatic and femoral nerves were severed. Aseptic precautions were observed in those animals which were to be kept for later use. In none of our experiments did infection of the muscles result. The skin suppurated in a few cases due to post-operative infection, but this seemed in no way to affect the muscle.

In the two experiments (p. 505) in which the changing volume of a limb after denervation was to be continuously recorded as well as its response to periodic doses of adrenalin, we connected the plethysmograph by means of a T-piece to two bellows, one large and one small. The slow changes were recorded on the larger one, while the little one (deflated) was clamped off. When the time came for injection, the clamp was removed from the small bellows tube, the latter bellows being slightly inflated by a small compression of the larger bellows, then the tube leading to the large bellows was clamped. The small bellows was thus prepared to register a small volume change in the limb. After the injection effects were finished, the clamp on the large bellows tube was removed, the small bellows was deflated by forcing the air into the large bellows and then clamped off. By this method no air was lost during the experiment.

RESULTS

Response after recent denervation. The peripheral effect of adrenalin was compared with the total "gangliar peripheral" effect in fifteen cats by studying the volume changes in a denervated limb simultaneously with those in a normal limb (2). The response of the denervated limb was predominantly constriction, although there was a short period of dilatation which usually occurred at the time of the blood pressure rise. Except in a few instances this was undoubtedly a passive effect. In these the dilatation persisted for a short time during the blood pressure fall and came earlier than that in the normal limb (fig. 1, *A*). This dilatation occurred only from small doses of adrenalin, a small increase in the adrenalin being sufficient to obliterate all but a slight passive effect (fig. 1, *B*). On the other hand more than ten times the dose of

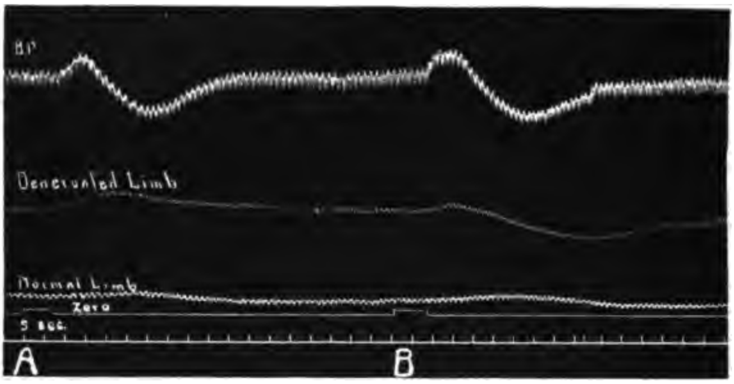

Fig. 1. Small active dilatation (A) of a denervated limb which occurs when 0.2 cc. of adrenalin, 1: 100,000 is given disappears when a slightly larger dose 0.4 cc. of the same solution, (*B*) is injected. Although the bellows for the normal limb was less sensitive, it does show that the maximum dilatation of the normal limb coincides with the maximum fall in blood pressure while the dilatation of the denervated limb does not coincide. Cat, 3.3 kgm. (Reduced one-half)

adrenalin was required to produce constriction in the normal limb as compared with that for constriction in the denervated limb, e.g., constriction in the denervated limb always occurred with doses of about 0.2 cc. to 0.4 cc., 1: 100,000 adrenalin or less, while from 0.3 cc. to 1.0 cc. 1: 10,000 adrenalin was necessary to produce a similar result in the normal limb.

Cutting the nerves to the limb must produce the result described either by removing the influence of the gangliar dilator mechanism or by modifying the blood vessels themselves so that they do not respond through the medium of the peripheral mechanism. From Gruber's work it appears that after some time has elapsed the dilator response to adrenalin develops in the denervated limb. He assumes that this is due to a recovery of tone. In order to test this theory we conducted the following experiments.

After both the sciatic and femoral nerves of one hind limb were dissected out and secured by loose ligatures, the limb was placed in a plethysmograph tube connected to the double bellows system described above. The nerves were severed and the change in volume of the leg registered every five minutes during the remainder of the animal's life. Every hour the response to a depressor dose of adrenalin was determined. In this way the adrenalin reaction could be studied in direct relation to the condition of relaxation or contraction of the vessel walls.

In the first experiment of this kind the animal (cat, 1.8 kgm.) was anaesthetized with ether and lived for eight hours. The limb dilated at an almost uniform rate for the first five hours after the nerves were cut. Dilatation became slower during the sixth hour and had completely stopped at the end, from which time the volume of the limb remained the same until the eighth hour, when the animal died. The blood pressure remained fairly good until a short time before death. The dose of adrenalin used for testing was 0.2 cc., 1: 100,000. Throughout the experiment this produced a fall in blood pressure, preceded by a slight rise. The limb responded by a short dilatation (which may easily have been due to the preliminary blood pressure rise) followed by a more prolonged constriction until the end of the sixth hour when active dilatation appeared. In other words while the limb was in the process of dilating as a result of denervation adrenalin caused constriction, but when the dilatation from this cause was complete a small amount of active dilatation occurred from adrenalin.

In a second experiment where urethane was given, the cat (2.2 kgm.) lived thirty-three hours. The maximum dilatation was reached between the sixth and seventh hours. It did not remain long at this level, but constriction soon began, continuing gradually until the twenty-second hour, when it ceased. It remained at this level for the next eight hours. The amount of this remaining dilatation was about one-fifth of the maximum. The dose of adrenalin in each instance was 0.5 cc., 1: 100,000. This usually produced a fall in blood pressure, which was preceded by a rise. During the first five hours adrenalin produced dilatation and constriction of the limb, the dilatation appearing to be largely passive. At the sixth and seventh hours the dilatation became more active and from that time onward the dilator reaction to adrenalin was more pronounced. This was undoubtedly due in part at least to active stimulation, although there was considerable variability in the curves, sometimes the constriction being more pronounced and the dilatation more passive (fig. 2). On the whole it may be said from the two experiments that active dilatation of a denervated limb in response to adrenalin becomes more prominent after the relaxation resulting from denervation has ceased.

In the above experiment we found that a large part of the dilatation resulting from denervation had been recovered from in eighteen hours and that there was little change for the next twelve hours. At this time if the nature of the reaction depends on the condition of tone in the vessels, adrenalin should give good dilatations. In addition to the experiment just described we tried two others. One

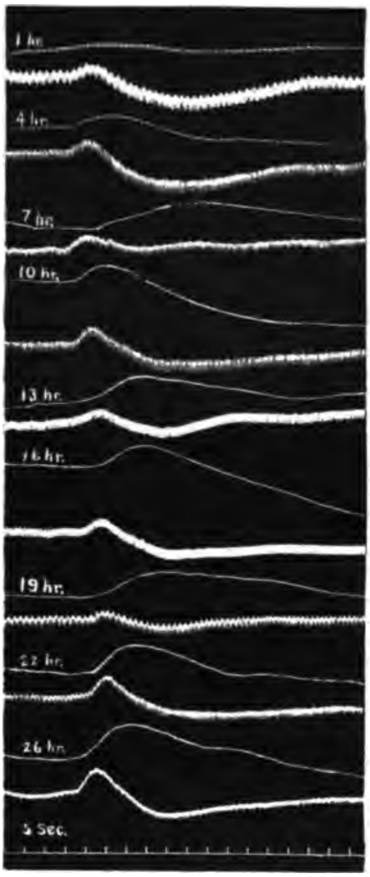

Fig. 2. The response of a denervated limb to a depressor dose of adrenalin during "atonic" and "tonic" conditions. The hours represent the length of time after cutting the nerves. The period of maximum dilatation was reached between the sixth and seventh hours. Up to that time the vessels may be considered "atonic;" after the seventh hour they may be considered "tonic;" 0.5 cc., adrenalin 1:100,000 was injected in each case. The upper record at each hour represents limb volume, the lower is blood pressure. Cat, 2.2 kgm. Urethane. (Reduced one-half)

hind limb was denervated in each of two cats. Eighteen hours later the animal was again anaesthetized with ether and a study made of the adrenalin response, with the following results:

Cat, 2.2 kgm., 0.3 cc., 1: 100,000 adrenalin caused a similar amount of dilatation in both the normal and denervated limbs. Doses of 0.5 cc. to 1.0 cc., 1: 100,000 adrenalin produced either constriction alone or else dilatation and constriction in the denervated limb. Larger doses produced marked constriction in the same limb. Doses as large as 5.0 cc., 1: 100,000 still produced dilatation in the normal limb, moreover these dilatations were much more pronounced than any resulting in the denervated limb. It took 0.8 cc., 1: 10,000 adrenalin to cause a reversal in the normal imb and then it was not complete, dilatation preceding the constriction.

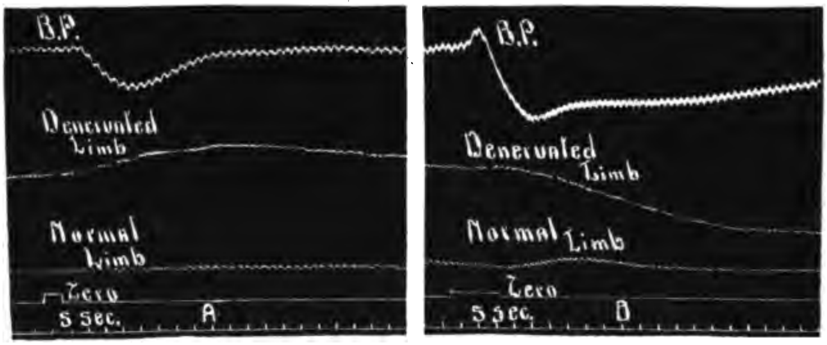

Fig. 3. *A*. Marked active dilatation of a denervated (18 hr.) limb with a small dose of adrenalin 0.2 cc., 1: 100,000. No effect in the normal limb. *B*. Constriction of the same denervated limb with 1.5 cc., 1: 100,000 adrenalin; dilatation of the normal limb. Cat, 2.6 kgm. (Reduced one-half)

Cat, 2.6 kgm., 0.2 cc., 1: 100,000 adrenalin caused a marked dilatation in the denervated limb, but no effect in the normal limb (fig. 3, *A*). Dilatation in the denervated limb, occurred with doses as large as 1.0 cc., 1: 100,000 but 1.5 cc. of the same concentration caused constriction (fig. 3, *B*). Dilatations were not produced in the normal limb until 0.3 cc., 1: 100,000 adrenalin was injected. Dilatation in this limb resulted from doses as large as 0.5 cc., 1: 10,000 adrenalin; however, 0.7 cc. of the latter concentration caused a reversal.

In both experiments the range of dosage for dilatation in the denervated limb was small while quite large amounts of adrenalin were required to bring about reversal in the normal limb. It seems from these experiments that tone may play a part in the response of a denervated limb to small doses of adrenalin. Moreover it appears that the

TABLE 1

A comparison of normal and denervated limbs

ANIMAL	WEIGHT	DURATION OF DENERVATION	DOSE	RESPONSE OF NORMAL LIMB	RESPONSE OF DENERVATED LIMB
	kgm.	*days*	*cc.*		
1. Cat	2.4	7	0.6 A	Dilatation*	Dilatation
			1.0 A	*Dilatation and constriction*	Dilatation
			0.2 B	*Marked constriction*	Dilatation
			0.5 B	*Very marked constriction*	Dilatation
			1.0 B	*Very marked constriction*	*Dilatation and constriction*
2. Cat	3.0	14	0.3 A	Dilatation	Dilatation
			0.4 A	*Constriction*	Dilatation
			0.7 A	*Constriction*	*Constriction*
3. Cat	2.2	15	0.4 A	Slight dilatation	Dilatation
			0.5 B	Slight dilatation	*Marked constriction*
			1.0 B	*Slight constriction*	*Marked constriction*
4. Dog	14.0	22	1.0 A	Dilatation	Dilatation
			1.6 A	Marked dilatation	Marked dilatation
			2.5 B	*Dilatation and constriction*	*Dilatation and constriction*
5. Dog	6.2	31	*0.2 A*	*Nothing*	Dilatation
			0.5 A	*Dilatation and constriction*	Dilatation
			0.2 B	*Dilatation and constriction*	Marked dilatation
			0.5 B	*Very marked constriction*	*Marked dilatation and marked constriction*
6. Dog	5.6	39	0.2 A	Slight dilatation	Slight dilatation
			1.5 A	Dilatation	Dilatation
			5.0 A	*Dilatation and constriction*	Very marked dilatation
			1.0 B	*Dilatation and constriction*	*Dilatation and constriction*

* Unless otherwise stated dilatation means active dilatation.
A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.

peripheral mechanism has a much more limited action than the "gangliar-peripheral" mechanisms when taken together.

After denervation of greater duration. Animals (six dogs and three cats) were studied which had had the sciatic and femoral nerves severed in one limb from seven to thirty days before. It can be seen from the following table (table 1) that although the lapse of a week in most cases renders the peripheral dilator mechanism more effective (see figs. 4 and 5), a greater amount of time does not materially increase the

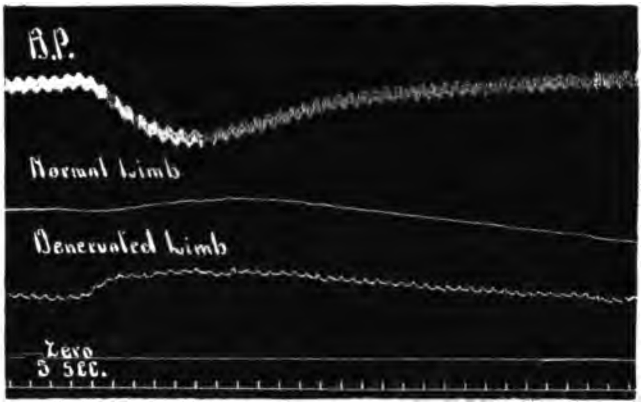

Fig. 4. Dilatation of the hind limb of a cat (2.4 kgm.) to 0.2 cc., adrenalin, 1: 100,000, seven days after denervation. (Reduced one-half)

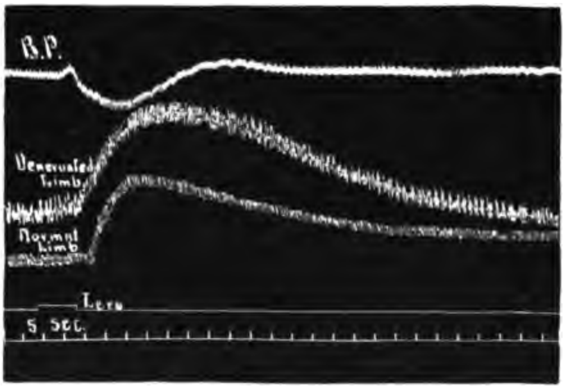

Fig. 5. Dilatation of the hind limb of a dog (14 kgm.) to 0.8 cc. adrenalin, 1: 50,000, twenty-two days after denervation. (Reduced one-half)

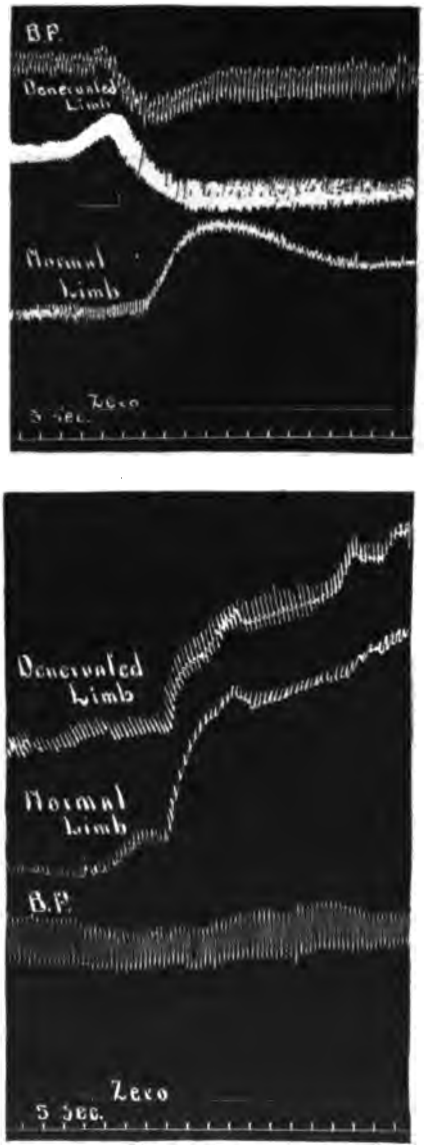

Fig. 6. Reversal of the adrenalin response in a freshly denervated limb by perfusion. Upper record—circulation to limbs intact, 1.0 cc. adrenalin, 1: 10,000 injected into the jugular vein. Lower record—limbs perfused, 2.0 cc. adrenalin, 1: 10,000 injected into the perfusion fluid. Dog 24 kgm. (Reduced one-half)

TABLE 2

Comparison of normal and denervated limbs before and after perfusion

ANIMAL	WEIGHT	DOSE OF ADRENALIN	NORMAL LIMB	DENERVATED LIMB
	kgm.	*cc.*		
7. Dog	17.0	0.5 A	Dilatation	
		0.3 B	Dilatation	
		0.7 B	Constriction	
		1.0 A	*Dilatation*	
		1.0 B	*Dilatation and constriction*	
8. Dog	15.0	1.3 A	Dilatation and constriction	Small dilatation and marked constriction
		0.5 B	Marked dilatation and small constriction	Marked constriction
		0.7 A		*Marked dilatation*
		0.4 B		*Dilatation and constriction*
		3.0 B		*Pure constriction*
9. Dog		0.5 A	Dilatation	Constriction
		0.4 B	Marked dilatation	Marked constriction
		0.7 B	Dilatation and constriction	Marked constriction
		1.0 A		*Dilatation*
		4.0 B		*Marked dilatation*
		1.0 C		*Dilatation and constriction*
10. Dog	7.5	0.4 A	Dilatation and constriction	Dilatation and constriction
		4.0 A	Dilatation and constriction	Marked constriction
		0.5 A	*Dilatation*	*Dilatation*
		1.0 B	*Constriction*	*Constriction*
11. Dog	24.0	0.5 B	Dilatation	Constriction
		2.5 B	Marked dilatation	Marked constriction
		4.5 B	Dilatation and constriction	Marked constriction
		1.0 B	*Marked dilatation*	*Dilatation*
		5.0 B	*Dilatation and constriction*	*Dilatation and constriction*

A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

Limb perfused where italics are used, injections in that case into the perfusion fluid, otherwise into the jugular vein.

effect. In most cases the constrictor mechanism had become less sensitive as compared with that in the normal limb (see animals 1, 2 and 5, table 1). On the other hand, occasionally the dilator mechanism was easily fatigued so that after a few doses the dilator response disappeared or was considerably decreased.

TABLE 3

*Comparison of perfused limbs of animals in table 1**

ANIMAL	WEIGHT	DOSE	NORMAL LIMB	DENERVATED LIMB
	kgm.	*cc.*		
4 Dog denervated 22 days	14.0	2.0 A	Dilatation	Dilatation
		0.4 B	Dilatation	Dilatation
		1.5 B	Dilatation and constriction	Dilatation and constriction
5. Dog denervated 31 days	6.2	0.05 A	No effect	No effect
		0.1 A	Small constriction	Small constriction
		0.5 A	Dilatation and constriction	Dilatation and constriction
		0.2 B	Dilatation and marked constriction	Dilatation and small constriction
		0.2 B		Marked dilatation
		0.5 B		Very marked dilatation
		1.0 B		Very marked dilatation
		0.5 C		Marked dilatation
		0.8 C		Dilatation and constriction

* Injections into the perfusion fluid.
A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

The dilatation of the denervated limb was no better developed in these animals than in some of the responses from a limb denervated but a few hours before (see fig. 2; 7 hr., 19 hr.). However the dilatation was more constant in occurrence and resulted from a greater range of doses. From the very fact that dilatation quite often takes place in the denervated limb from doses larger than those necessary to produce reversal in the normal limb, it seems that a change has taken place in the myoneural junctions. The constrictor junctions must have lost in sensitiveness or the dilator junctions have gained.

Response of perfused limbs. We have obtained dilatation of both normal and denervated limbs from the injection of adrenalin into the fluid which was perfusing them. A comparison of the perfused normal and denervated limbs injected in this way should help to explain the peripheral dilator mechanism.

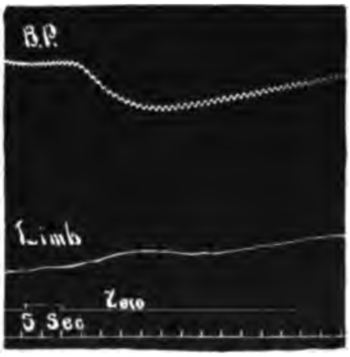

Fig. 7

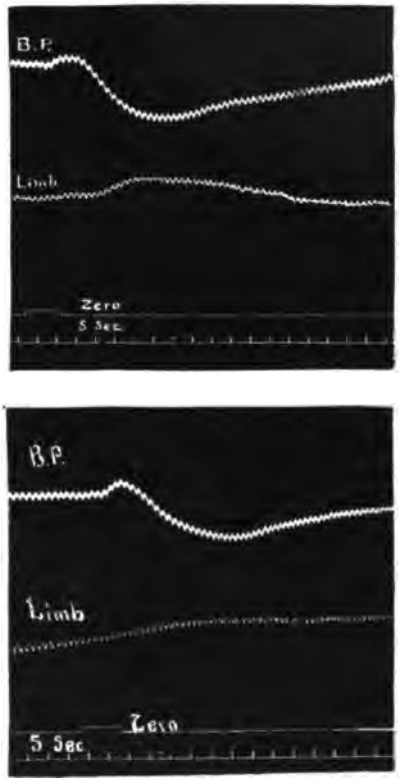

Fig. 8

Fig. 7. Dilatation of a perfused hind limb of a cat by the action of a depressor dose of adrenalin (0.6 cc., 1: 100,000) upon the gangliar portion of the dilator mechanism. Cat 2.4 kgm. (Reduced one-half)

Fig. 8. Dilatation of a hind limb produced by a depressor dose of adrenalin acting upon the gangliar portion of the dilator mechanism. Upper record—response of the hind limb to 0.4 cc. adrenalin, 1: 100,000 injected into the jugular vein, circulation intact. Lower record—response of the same limb to 0.6 cc. adrenalin, 1: 100,000 injected into the jugular vein. (Reduced one-half)

By perfusion of a recently denervated limb an immediate change in the response to adrenalin is brought about, so that dilatation instead of constriction is easily produced (fig. 6 and table 2). This change is similar to that occurring in a denervated limb with normal circulation several hours after denervation (figs. 2, 3, 4, 5 and table 1). The peripheral response to adrenalin in perfused normal and denervated limbs is essentially the same when carried out simultaneously in one animal (animals 10 and 11, table 2). On the other hand there is greater variability in the response of a perfused limb which has been denervated for several days. In one case the constrictors were easily fatigued so that after a few doses of adrenalin they could not again be brought into action except by a dose of 0.8 cc., 1: 1,000 (animal 5, table 3). In another case even perfusion did not bring about dilatation in an animal which had shown no active dilatation with intact circulation (dog, limb denervated eight days). The normal limb gave dilatation before and after perfusion but this is an exceptional case in our experience.

"Gangliar" dilatation from depressor doses. Gruber (9, p. 311) failed to obtain dilatation of a perfused limb from the injection of depressor doses of adrenalin to the general circulation. He infers that the gangliar effect is produced only by pressor doses. We have been able to show in two experiments that depressor doses of adrenalin can bring the gangliar mechanism into action. In both animals a slight increase in the dose was necessary, but the blood pressure response was a pure fall or else a slight rise and decided fall. The animals were cats weighing 2.4 kgm. and 3.0 kgm. In the first, 0.6 cc., 1: 100,000 was required after perfusion (fig. 7). In the second, 0.4 cc., 1: 100,000 caused dilatation before, while 0.6 cc., 1: 100,000 was required after perfusion (fig. 8). When perfusion had gone on for some time even larger doses of adrenalin were required to produce dilatation.

DISCUSSION

The relation of tone to the reversal of adrenalin effects. Recognizing the fact that adrenalin may cause dilatation through both gangliar and peripheral action, we are confronted with the question as to the normal site of dilator action. It has been shown that cutting gangliar connection with the limb in a majority of cases prevents the dilatation of that part. Gruber (9, p. 307) maintains that this is due to a loss of tone in the vessels. In order to understand the development of the tone theory, we should first consider the work of Cannon and Lyman

(10) who were the first to suggest this interpretation for the opposite effects of depressor doses of adrenalin. Their view was reached by the exclusion of other possibilities, viz., (1) central source, (2) blocking of vasoconstrictor impulses, (3) stimulation of vasoconstrictor and vasodilator nerve endings. Their exclusion of the third possibility was on account of the meagre evidence for the existence of vasodilator nerves in the sympathetic system. They found that the blood pressure response was changed to a rise if the tone had been lowered sufficiently by overheating, separation from the central nervous system or by extreme action of the depressor nerve. They attributed vasodilation and vasoconstriction to opposite actions of adrenalin according to the state of the muscle—relaxation when tonically shortened, contraction when relaxed.

Gruber's conclusions were reached because of his inability to obtain dilatation in a freshly denervated limb and the recovery of the dilator response in a limb a few days after denervation. He attributed the reappearance of the dilator reaction to a restoration of tone. It might also be due to a loss in sensitiveness of the constrictor myoneural junctions.

Let us consider, first, the question of tone. In all of our experiments with recently denervated animals the reactions to adrenalin were studied within thirty minutes after denervation and were continued for one or two hours. We have shown above that the maximum dilatation is not reached until the sixth hour after denervation so that those studies were made during the period of steady relaxation. Within this time the usual adrenalin response is constriction, afterwards the reaction begins to reverse (fig. 2). It is not that the vessels have suddenly dilated to their limit and cannot expand further, because they only gradually reach this stage after six or seven hours. Moreover they do not appear to dilate to the limit at any time as a result of denervation because while they are in this state of maximum relaxation, depressor doses of adrenalin often cause further dilatation (fig. 2). We may draw the conclusion that while relaxation is going on the vasodilator myoneural junction is not so easily brought into action and that the constrictor effect therefore predominates.

The state of relaxation seems to affect only the adrenalin receptive substance. Active dilatation of a denervated limb in which the vessels are relaxing can easily be produced by a substance from ox pituitaries (fig 9). In the same animal depressor doses of adrenalin usually caused constriction of the denervated limb (fig. 1).

After denervation of greater duration. A few days after cutting the nerves to a limb, the latter has regained its power to dilate in response to adrenalin so that it does as well as the normal limb. This might be explained by the recovery in tone, but a large part of the tone has been recovered within twenty-four hours, so that the reaction at the twenty-fourth hour should not differ much from that several days later. But it does differ in this respect that in denervations of longer duration it requires much larger doses of adrenalin to cause constriction; in other words, there is a larger range of dosage producing dilatation. In fact a larger dose than that required for the normal limb is needed to bring about reversal in the majority of cases (table 1). Gruber (9, p. 310) also found this to be true. One can interpret this either as a loss in sensitiveness of the constrictor junctions or a gain in sensitiveness of the dilator junctions. The tone theory, however, does not appear to account for this point.

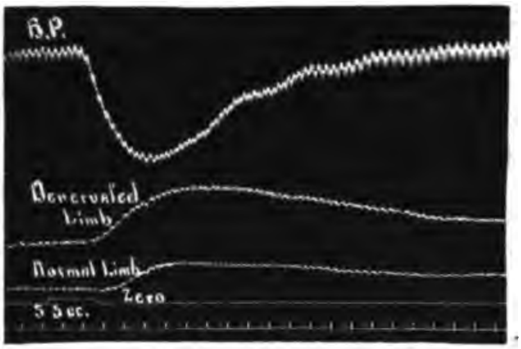

Fig. 9. Dilatation of a freshly denervated limb, produced by a depressor substance obtained from pituitary glands. Cat. (Reduced one-half)

In regard to the question of variation in sensitiveness of the myoneural junctions we have the work of Elliott (12), which indicated that all muscles thrown into contraction by adrenalin have their irritability to this substance increased by denervation. However we have no proof that dilator junctions would be thus affected.

Effects of adrenalin in perfused limbs. A number of investigators have studied the response of various perfused organs to adrenalin with variable results. This would be one of the best methods of proving the existence of vasodilator nerves in the sympathetic if active dilatation could be so obtained.

Employing the change in rate of venous outflow to indicate the vasomotor response Salvioli (14) and Brodie and Dixon (15) obtained only constriction in the hind limb when adrenal extract or adrenalin was added to the perfusion fluid. The latter experimenters found this to be true even in limbs which had been denervated two or three months before. Pari (16) repeatedly obtained an increased outflow from the limb in one experiment when a perfusion of 1:500,000 adrenalin was used, but he inferred that this was due to decomposition products.

Langendorff (17) from his results with rings of coronary arteries concluded that they possessed sympathetic vasodilators which were stimulated by adrenalin. His results were confirmed by Cow (18) and Park (19). Brodie and Cullis (20) from experiments upon perfused hearts concluded that the main cause of adrenalin dilatation was the excitation of vasodilator "nerve-endings."

Langlois and Desbouis (21) obtained constriction in the lung vessels with large doses, 1.0 mgm., and dilatation with small doses, 0.05 mgm. Similar results on perfused lungs were described by Tribe (22).

Other organs have given dilatation from dilute adrenalin perfusing them. For instance, the kidney and the intestine have been found by Ogawa (23) to react in this way. But so far as we know the limb has not been found to react thus when perfused except in the one experiment of Pari (16) and in experiments by Ogawa (23) on the rabbit in which he sometimes obtained dilatation following constriction, but never primary dilatation.

We found it easy to produce dilatation by the injection of adrenalin into the fluid perfusing a limb. Whether the nerves had been cut or not seemed to make no difference in the reaction (table 2).

Why should perfusion reverse the reaction of a denervated limb? Does it mean that perfusion of vessels which were previously relaxing causes them to begin to contract and thus produces the reversal? That might be the condition in perfusion with low pressure (20 mm.) but in a number of our experiments we have doubled or tripled the pressure without materially reducing the dilator response to adrenalin. Moreover Tribe (22) found in the perfused lung that with high pressure it was easier to obtain dilatation than constriction.

Another observation which suggests an explanation of the results just described is the increase in the range of doses of adrenalin which will cause dilatation in both normal and denervated limbs. The interpretation which this seems to suggest is that perfusion renders the constrictor myoneural junction less sensitive or the dilator junctions more sensitive. We have found that it takes much larger doses of adrenalin to bring about constriction in a perfused limb than it did while the circulation was intact, whether it be a denervated limb or one with nervous connections (table 2). For example: whereas 1.0 cc., 1:10,000 adrenalin injected into the jugular vein before perfusion caused constriction in the denervated and dilatation in the normal limb, 2.0 cc., 1:10,000 (a dose more than four times as great, considering the limited circulation of the perfusion fluid) injected into the perfusion fluid caused marked dilatation in both limbs (fig. 5). Mechanical effects from the injection were compensated for by a simultaneous withdrawal of an equal quantity of perfusion fluid.

The work of Meyer (13) supports the idea that Ringer's solution modifies the sensitiveness of blood vessels to adrenalin. He found that artery rings kept for some time lost their sensitiveness to adrenalin from day to day and after it had disappeared an opening shock still produced contraction. His results might be due to the changed medium in which the preparations were kept rather than to denervation.

Dilatation from the stimulation of "gangliar" and "peripheral" mechanisms. Before we enter into the discussion of the relative importance of the "gangliar" and "peripheral" mechanisms we wish to call attention to the results of Gruber (9, p. 311), in which he failed to obtain dilatation of a perfused limb from the injection of a small dose of adrenalin into the general circulation. Because the same dose caused dilatation in the intact limb he infers that the dilatation from small doses must be due to peripheral instead of gangliar action. He says:

> If adrenalin exerted its influence entirely through a vasodilator center, it should produce the same results in these two cases where the only difference in the conditions of the limbs is that one has and one has not the circulation intact.

This is a very serious difference and might easily account for the increase in the dilator threshold. Oxygenated Ringer's solution or even oxygenated defibrinated blood cannot be expected to fulfil the function of normal blood in all respects and indeed this was not the only difference, for the occlusion of the abdominal aorta interferes with the circulation to the ganglia of the nerves supplying the limbs. The latter condition alone might necessitate a larger dose of adrenalin. If both of these conditions were operative, the dose required would probably in many cases be a pressor dose. However, we have been able to show in two experiments that depressor doses can bring the gangliar mechanism into action. These render unnecessary the assumption of peripheral action to account for dilatation resulting from small doses of adrenalin.

We are not in a position to say which is more important in producing dilatation normally, the "gangliar" mechanism or the myoneural junction. It has been possible in some animals to obtain the same amount of dilatation by the action of adrenalin upon the gangliar portion of the mechanism alone (limb perfused, adrenalin injected into the jugular vein) as occurred from the injection of the same quantity when the circulation of the limb was intact. In many cases, however, larger doses are required to produce equal response in the limb when only the gangliar mechanisms are affected as compared with the condition where both gangliar and peripheral portions might be brought into action. This may easily be attributed to the reduced circulation to the ganglia brought about by clamping the aorta high in the abdomen, but the fact that the peripheral dilator mechanism can be brought into action rather easily under many circumstances indicates that it may well be as important as the gangliar dilator mechanism. At any rate we seem justified in concluding that sympathetic vasodilators to the limb exist and that they are sensitive to adrenalin at the "gangliar" and "peripheral" ends.

We wish to thank R. S. Lang for assistance in this research.

SUMMARY

1. While a limb is dilating from denervation adrenalin produces an increase in volume with difficulty, but while the reverse change is taking place the dilator effect of adrenalin begins to reappear.
2. After denervation of a limb, of greater duration, the dilatation from adrenalin occurs from a greater range of doses than is the case in the normal limb.
3. The peripheral action (dilatation) becomes similar in both normal and denervated limbs after perfusion. Under these conditions also dilatation occurs with a greater range of doses.
4. Depressor doses of adrenalin can cause dilatation of a limb by action on the gangliar mechanism.
5. Adrenalin acts on both "gangliar" and "peripheral" mechanisms in producing dilatation of the hind limb.

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CONSTRICTION FROM ADRENALIN ACTING UPON SYMPATHETIC AND DORSAL ROOT GANGLIA

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In the preceding research it has been shown that adrenalin can produce dilatation in a limb by acting upon a "peripheral" mechanism as well as upon a gangliar mechanism. We have been able to show that the constrictor action of this hormone is not confined to the myoneural junction. Although the gangliar response is not easily obtained, it has been found often enough to draw our attention. The methods employed were those described in preceding researches.

All experiments showing constriction from gangliar action must necessarily be those in which the organ tested is completely cut off from the general circulation in order to prevent the peripheral action of adrenalin.[1] Perfusion experiments in which anastomoses to the organ are cut off, satisfy this condition.

Constriction of the limb. Six animals out of nineteen furnished evidence of gangliar constriction in the hind limb. One dog (16 kgm.) and one cat (3 kgm.) gave constriction followed by dilatation when adrenalin was injected into the jugular vein; the first with a dose of 4 cc., 1: 20,000 adrenalin, the second with a dose of 5 cc., 1: 5,000 adrenalin. In each animal both sympathetic and dorsal root ganglia were intact. On the other hand similar experiments with six dogs and three cats gave no constriction although the usual dilatation could be obtained.

From sympathetic ganglia. Two cats gave positive evidence of a constrictor action of these ganglia by the direct application of adrenalin to them. In the first, a 1: 100,000 adrenalin solution produced only dilatation while a 1: 10,000 solution caused steady marked constriction. In the other a 1: 1,000 solution caused a dilatation followed by constriction. Three animals gave no constriction. The first (a cat) was tried by dropping adrenalin upon the sympathetic ganglia. On the last two (dogs) the dorsal root ganglia had been removed, adrenalin being given by the jugular vein.

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[1] Salvioli (Arch. ital. de biol., 1902, xxxvii, 384) perfused the limb of a dog, with the nerves intact. Adrenal extract was injected into the jugular vein and the volume change in the limb was studied by the venous outflow. He usually obtained no change in the flow but occasionally there was a small decrease in the outflow. This was believed to be due to the escape of adrenal extract into the limb because the decrease was not synchronous with the rise in blood pressure; in fact the pressure had returned to normal before the limb changed.

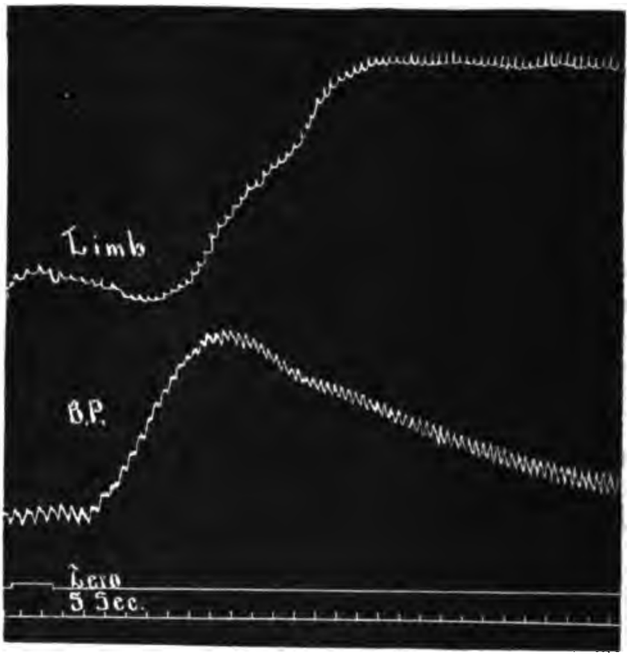

Fig. 1. Constriction and dilatation of a perfused limb from the injection of 4 cc. adrenalin, 1: 5,000 into the jugular vein. All sympathetic ganglia supplying the limb had been destroyed. Dog 21.6 kgm. Reduced ⅓.

From dorsal root ganglia. Of the animals (seven dogs) in which the sympathetic ganglia to the perfused hind limb had been destroyed, only one responded by constriction when adrenalin was injected into the general circulation (fig. 1). Direct application of adrenalin to the dorsal root ganglia in one of two cats caused constriction in the hind limb (fig. 2). In almost all of the animals studied whether giving gangliar constriction or not, dilatation from adrenalin was obtained.

We may say, in general for the hind limb, that the effect of adrenalin on the ganglia is preëminently dilator and that the constriction from this source is insignificant.

Constriction of the intestine. Constriction of a gangliar source was more common in the intestine than in the limb. A response of this sort was obtained in six out of thirteen animals. Moreover the number of constrictions obtained in the same animal was much greater in the case of the intestine than in the experiments with the limb. In the latter there would often be only one or two constrictions throughout the whole experiment.

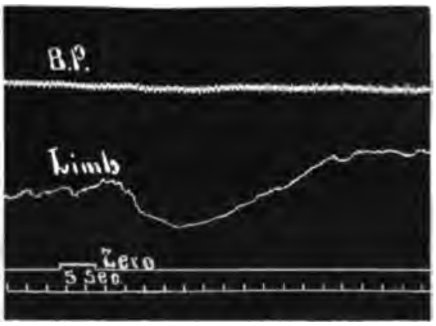

Fig. 2. Constriction of the hind limb resulting from the direct application of 1: 1,000 adrenalin to the lower lumbar dorsal root ganglia. Dog 16 kgm. Reduced ½.

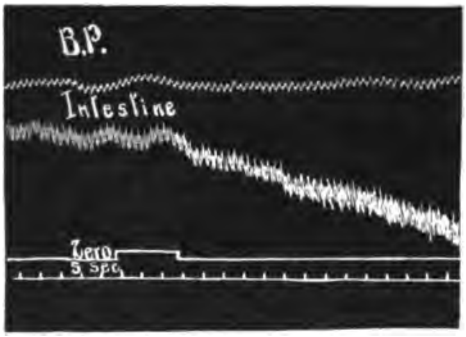

Fig. 3. Constriction of the intestine produced by direct application of 1: 1,000 adrenalin to the twelfth and thirteenth dorsal root ganglia. Dog 11 kgm. Reduced ½.

Three dogs whose splanchnic nerves had been cut gave positive evidence of gangliar constriction. The intestinal loop was perfused and the adrenalin was injected into the jugular vein. Both constriction and dilatation occurred whenever the intestine responded by constriction.

Intestinal constriction was also produced by the direct application of adrenalin to the dorsal root and superior mesenteric ganglia.

In a cat although dilatation only had been produced by the application of 1:1000 adrenalin to the twelfth and thirteenth thoracic dorsal root ganglia in three instances, in a fourth the same concentration produced constriction followed by dilatation.

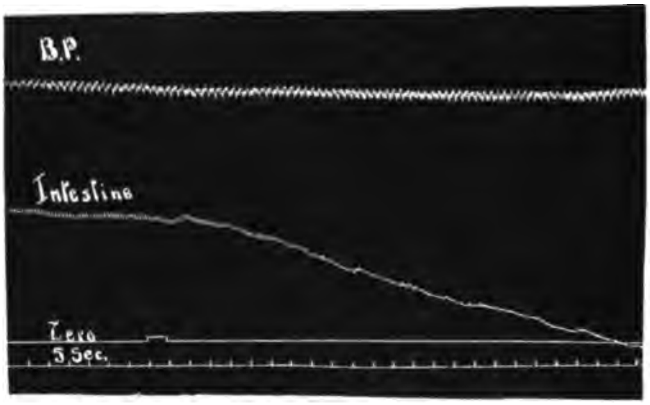

Fig. 4. Constriction of the intestine from direct application of 1: 1,000 adrenalin to the superior mesenteric ganglion. Dog. Reduced ½.

In a dog, a 1:1,000 solution produced dilatation alone, constriction alone (fig. 3) or constriction followed by dilatation.

Marked constriction of the intestine was caused in another experiment by treating the superior mesenteric ganglion with 1:1,000 adrenalin chloride to which a little pure adrenalin had been added (fig. 4).

Additional evidence that the superior mesenteric ganglion is a source of constriction was obtained in one animal by the use of nicotine. Before nicotine, adrenalin caused constriction followed by dilatation of the intestine. Intravenous injection of nicotine ruled out both the constriction and dilatation.

Thus the gangliar effect of adrenalin as far as the intestine is concerned is largely dilator, although it is sometimes a source of constriction.

SUMMARY

1. Adrenalin occasionally produces constriction in the hind limb by its action upon the sympathetic and dorsal root ganglia.
2. Constriction of the intestine is sometimes produced by adrenalin acting upon the superior mesenteric and dorsal root ganglia.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 92, AND FROM THE ANATOMICAL LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL, NO. 62.

THE MULTIPLE SENSORY ACTIVITIES OF THE SO-CALLED RHINOPHORE OF NUDIBRANCHS

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PRELIMINARY

A striking feature of nudibranch molluscan morphology is the pair of short, robust dorsal tentacles which are commonly perfoliate or ringed and which in many species are retractile. These distinctive tentacles are known as "rhinophores" and it is tacitly assumed that they are, as the name indicates, specialized organs of the olfactory sense.

The only evidence that would at all favor the assigning to these structures of an exclusive or predominating olfactory function has been presented by Graber ('89). This experimenter brought oil of rose near the head of Chromodoris elegans and observed the withdrawal of the rhinophores to be quicker and more vigorous than that of the oral tentacles. It is significant, however, that Graber states emphatically that the post-branchial region is the most sensitive part of the body. By whom the word "rhinophore" was coined and in what sense it was first applied is not clear; malacologists to whom I have referred this query are unable to trace its origin.

It thus appears that the propriety of the convenient term rhinophore lacks appropriate experimental support. For this reason nudibranchs were subjected to experimentation designed to test their sensory potentialities ('17).[1] Unless otherwise stated the present account deals with the Bermudian Chromodoris zebra Heilprin.

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[1] This work was made possible through the hospitality of Professor E. L. Mark, Director of the Bermuda Biological Station, and the liberality of the trustees of the Humboldt Fund of Harvard University.

OBSERVATIONS[2]

Tactile stimulation. When a rhinophore is touched lightly with a glass rod it is jerked back precipitately within its protecting collar. The sensitivity of the rhinophore to gentle stimulation is astonishing and the explosive type of response is, within wide limits, independent of the strength of the stimulus. Fatigue comes on but slowly, responses of somewhat diminished intensity being readily obtained after fifty successive stimulations at ten-second intervals.

The oral tentacles, gill plumes and the general body-surface all respond to tactile stimulation. It is rather unsatisfactory to list the several regions of the body in the order of their sensitivity, for the types of responses are not all comparable. It appears, however, that the so-called rhinophore is the most sensitive part of the body to this kind of stimulation and considerably more so than the oral tentacles.

Thermal stimulation. The head region and especially the oral tentacles react distinctly to water at 40° to 50°C. applied with a pipet. The rhinophores, on the contrary, give faint and rather doubtful responses except to temperatures as high as 50°C.

Rheotrophic stimulation. I am informed by Dr. W. J. Crozier[3] that the rhinophore of Chromodoris is of prime importance in effecting orientation to water currents. Animals from which these structures have been removed do not orient at all, or do so with extreme slowness and hesitancy; when only one functional rhinophore is left, circus movements are simulated.

Chemical stimulation. Equal volumes of various chemical solutions were applied from a constant distance with a pipet; with care tactile stimulation by the stream can be avoided. Solutions of 1 M maltose, or sucrose, or M/2 lactose were without effect upon all parts of the body, although 3 M glycerin did evoke general responses. Several alkaloids had very weak effects or none at all. Alcohols and organic acids in concentrations of M/10 called forth strong general responses. The chlorides of the alkali metals Na, K, NH_4 and Li likewise stimulated the body in general, the rhinophores and oral tentacles, however, showing the greatest sensitivity. Solutions of substances which produce in man the taste sensations recognized as acid (HCl), bitter (picric acid), salty (KCl), and alkaline (KOH), were applied in various concentrations. All responses gradually weaken with increasing dilution, but the gills fail usually before other parts. There was some evidence that the oral tentacles were more sensitive to picric acid than were the rhinophores.

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[2] An extended account of the behavior and sensory physiology of Chromodoris is in preparation by the writer in collaboration with Dr. W. J. Crozier.

[3] Unpublished observations.

From the foregoing tests it becomes evident that the rhinophore is not only extremely sensitive to chemical stimulation of diverse sorts, but that this sensitivity is only second to, if indeed it does not equal, that of the oral tentacles, which from their position might be suspected *a priori* of a specialized gustatory or common chemical function.

Olfactory stimulation. Saturated solutions of various essential oils[4] were prepared by shaking with sea water. These solutions were applied gently by a pipet to the several regions of the body surface.

The rhinophores of Chromodoris react vigorously to such stimulation but, so far as one can judge from the dissimilarity of the responses, other parts of the body appear to be equally sensitive. When a drop of oil is held for some time midway between the rhinophores no response ensues. If the rhinophore or general body surface be touched gently with a drop of pure oil, the response is weaker than to a saturated aqueous solution; in this case the number of sense organs stimulated undoubtedly is a complicating factor, yet it suggests further that the response is one to an olfactory stimulus rather than to an irritative or "smarting" one.

Chromodoris was also tried with solutions in which marine invertebrates had partially decomposed. Such a test is undoubtedly complicated by the presence of certain chemical substances not of an odorous nature, yet it at least simulates the type of olfactory stimulation met by the animal in a state of nature.

Water contaminated by a dead crab or by the viscera of a holothurian (these solutions being decidedly odoriferous to the human sense of smell) stimulated strongly all regions of the external body surface. Water from decaying coral or Onchidium likewise evoked responses from the general body surface except the gills. On the contrary, another specimen of coral water which did not smell particularly strong, was found to stimulate the rhinophores and head region in general but not the rest of the body; the sensitivity of the several head structures was, however, equal.

Facelina goslingi possesses long oral tentacles and rhinophores. Its entire body surface, including the rhinophores, is responsive to tactile and chemical stimulation. The animal is exceedingly active, climbing the walls of a container and swimming on the surface film. It was found that merely holding a drop of oil near the body of a crawling animal did not provoke a response, whereas actual contact (eliminating tactile complications) would do so. When stimulated with solutions the non-retractile rhinophores react by a lashing withdrawal and more vigorously than do the oral tentacles; this is the only clear case recorded of a superior reactivity of this organ to odorous substances. Oil of pennyroyal, carbon bisulphide and anilin oil proved to be more efficient than the oils of bergamot, cassia, cloves, juniper and origanum.

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[4] Bergamot, cassia, clove, juniper, origanum, pennyroyal and thyme. Anilin oil and carbon bisulpide were used also.

Elysia crispa is a small nudibranch which also tries to swim on the surface film. When crawling on the substrate its anterior half often loosens its attachment and is elevated and waved about, the posterior half still locomoting the while; this attitude is favorable for detecting the effect of stimulation. To light touch alone there is a slight retraction of the extended body. The odorous oils are more stimulating; to bergamot, cassia, cloves, juniper, origanum, pennyroyal and thyme applied to the rhinophores the retraction is noticeably sharper and of greater amplitude. Carbon bisulphide and anilin oil are without effect; this is in decided contrast to the results on Facelina recorded in the last paragraph. The general body surface is likewise responsive to these olfactory agents and to ordinary chemical stimulation as well; to solutions of the essential oils in sea water, the head region was more sensitive than the remainder of the body.

Fiona marina, a nudibranch found in the floating gulf weed, Sargassum, is sensitive on its rhinophores and body to essential oils and to other general chemical stimulation.

DISCUSSION

There is nothing in the foregoing tests which specifically connects the rhinophores with olfaction. Rather they appear to respond to various sorts of sensory stimuli, and to share this sensitivity liberally with other regions of the body. In Facelina alone is there a sharper rhinophore reaction, and even in this case it is not certain that the vigor of response to supraminimal stimuli is the expression of greater inherent sensitivity.

We know nothing concerning the existence of diverse sensory endings in nudibranchs for the reception of chemical stimuli. Presumably they do not exist (cf. Smallwood, '12).[5] Under such conditions it is futile to speak of a sense of touch, taste, smell and so on. It is true that many invertebrates are capable of responding to a variety of sensory stimuli and that, furthermore, such responses are often more or less accurately adjusted, in a quantitative manner, to the quality, strength or frequency of the stimulating agent; yet the occurrence of differentially selective responses to definite stimuli is limited. In certain instances, to be sure, specific sense organs (e.g., eye; otocyst) are recognized which respond exclusively or chiefly to particular stimuli; more usually identical responses follow widely diverse sensory excitants. Much of the older results regarding the existence and localization of differential sensitivity is uncritical and untrustworthy.

There is no reason to believe that organs structurally uniform and capable of responding identically to a variety of qualitatively different stimuli, are able to analyze, thereby producing qualitatively distinct sensations. In other words, it can not be assumed that such an animal is capable of differentiating various stimuli intuitively, nor that as a functional adaption individual receptive elements, morphologically indistinguishable, have acquired the capability to analyze. Such assumptions are opposed to the principle of specific energies, which is by far the safest guide in the study of invertebrate sensory potentialities.

Histologically identical sense organs, however, due to topographical arrangement, may in a practical way chiefly serve physiologically distinct functions. Thus, certain receptors about the mouth are said to be useful mainly for proving food, those at the entrance of the respiratory chamber, for testing the quality of the respiratory medium, and so on. This method of logical assignment of function is not without danger; for example, the insect antenna has been persistently associated especially with olfaction, yet McIndoo ('14) finds that this classic example will not stand the test of experimentation.

If the foregoing conclusions be sound they are of interest in view of a recent contribution by Copeland ('18) who, reflecting the tone of a certain group of writers, reports on what is termed the olfactory reactions of marine snails. That the animals studied sought food through the action of a chemical sense located chiefly in the osphradium, although the external body surface was also responsive to a lesser degree, seems certain. But the contention is pressed further: that, since the osphradium responds to weak concentrations of oyster juice and the rest of the body to relatively high concentrations, the osphradium must be of an olfactory nature and the general body surface gustatory.

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[5] It is, however, not impossible that what is accepted in certain instances as a specific form of nerve terminal, such as a free nerve ending, might upon close study be amenable to division into morphologically distinct types. On the contrary, the really significant and fundamental qualitative differences (histologic or chemical) could easily be so subtle that our crude methods must ever fail to detect them. Furthermore, although it is perhaps true that even the simplest metazoans which have nerve terminals at all have them somewhat differentiated, the fact remains that in this field as a whole the anatomic evidence lags.

This conclusion by no means follows. In the absence of differential receptors a separation into smell and taste on the basis of sensitivity alone is no more defensible than to split into two categories the tactile sense of the human finger and back.

In man, to be sure, the diverse sensitivity of smell and taste to substances that stimulate both is one distinguishing characteristic (Parker and Stabler, '13), but there are other more germane criteria of a qualitative nature. Moreover, to argue by analogy from man to lower vertebrates with respect to these sensory activities is not especially hazardous, for the homologies of innervation and structure are not only certain but the one mechanism or the other can be eliminated experimentally with precision (by section of nerves, plugging of nostrils, etc., Sheldon, '11; Parker and Sheldon, '13). In invertebrates, however, where not even the existence of separate, appropriate end organs has been demonstrated, the argument by analogy exceeds the limits of legitimate deduction.

Finally, although such substances as fish juice or oyster juice combine elements of taste and smell for human sensibilities, we must distrust the capability of the invertebrates in question to analyze chemical stimuli as discrete sensations, and it would therefore appear safer to avoid referring in their cases to a sense of taste and smell at all (or even to a common chemical sense, since this term is preëmpted in vertebrate physiology). What such animals possess can perhaps be designated a "general chemical sense."

SUMMARY

Experimental evidence does not substantiate the popular impression that the nudibranch rhinophore is a specialized organ for detecting odorous substances. It is, rather, a generallzed organ responsive to a variety of sensory agents.

In the absence of differential receptors and the probable resulting failure to translate stimuli into discrete 'sensations,' it is safer to avoid terms like taste or smell, especially with the implication of any similarity to corresponding human sensations. Since we comprehend sensory processes only in terms of human experience, there is, however, no objection to retaining such convenient phrases as "olfactory stimulus."

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ACAPNIA AND SHOCK[1]

VIII. The Veno-Pressor Mechanism

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There must be some mechanism controlling the supply of blood from the veins to the heart and regulating the variations needed during rest and exercise. This mechanism must adjust the venous pressure to dilate the right heart during diastole, neither over nor under filling the ventricle.

The variations in the volume of the venous return required and provided are enormous. During bodily rest the venous pressure in man in the erect position rises scarcely above the axilla. During exercise, when the heart is pumping out of the venous system a greatly increased volume of blood, the venous column instead of being reduced is many centimeters higher than in bodily rest (1 and 29).

What is the controlling mechanism producing this rise? Is its action direct or indirect, primary or secondary? What is the influence, nervous, mechanical or chemical, which adjusts its activity to the general needs? Is the locus of stimulation central or peripheral? Undoubtedly the universal answer today would be included under: Regulation by the vasomotor nervous system.

The purpose of this paper is to present facts indicating that, in addition to indirect vasomotor nervous influences (2), *there is a peripheral chemical control of the volume of the venous return*, and reasons, based on previous papers from this laboratory, for holding that it is this chemical control which is the chief factor in the high venous pressure of muscular work and in the lowering of venous pressure induced by acapnia and itself causing the circulatory depression following anesthesia and surgical operation.

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[1] We use the term "shock" merely in the sense of "depression of vitality." Surgical shock is the depression following anesthesia and operation; traumatic that due to trauma, and toxaemic that incident to toxaemia. Even ten minutes of anesthesia and a minor operation may depress vitality to some extent. A major operation usually depresses greatly for hours or days. Our problem is the causes and modes of these depressions, i.e., shock in all degrees, small as well as great. Shock as a moribund or almost moribund state, the sense in which most investigators seem now to use the term, appears to us to be a conception which for purposes of investigation is both artificial and sterile.

The nature of the problem is seen when we compare the circulatory effects of muscular exertion with those of mental strain. The latter causes a very marked rise of arterial pressure—a rise of 30 or 40 mm. of mercury, and once even 80 or 90 mm. in several subjects has been observed in this laboratory during a mere lecture on the blood gases. We suppose this high arterial pressure to be an almost pure vasomotor nervous effect, aided perhaps by some adrenal or other internal secretion. There is however no rise of venous pressure—a fact which becomes highly significant when we consider that the abundant chemical stimulations, altered blood gases and especially increase of CO_2, involved in muscular effort are lacking in mental strain.

On the other hand physical exertion—running, stair climbing or riding a stationary bicycle—sufficient to cause a rise of 30 to 40 mm. of mercury in arterial pressure always induces also a rise of the venous pressure so marked that the hand and arm may be lifted many centimeters above the axilla before the veins collapse. Even when every allowance is made for the accessory parts played during physical work by respiration and by muscular movements in pressing the venous blood onward toward the heart, there seems to be in these facts ground for suspecting the existence of some factor in the circulation acting as a powerful accessory to the vasomotor nervous system in accelerating the venous return, and regulated by chemical rather than by nervous influences (cf. Boothby, 3).

As regards the depression of the circulation induced by anesthesia (4) and surgical operation and by acute disease,—conditions in which the CO_2 content of the blood varies in the opposite sense to that occurring in muscular work,—the evidence brought forward in previous papers from this laboratory points clearly to a lowering of venous pressure and decrease of the venous return as the primary factor (1), (5), (6). And yet in such conditions the vasomotor nervous system is not inactive but rather exerted to increasing and finally maximal effort in constricting arterioles in the endeavor to maintain arterial pressure. It thus strives in normal fashion to compensate the decreasing output of the heart. It acts exactly as after hemorrhage (7).

In harmony with this is the fact that the circulatory effects of even slightly excessive respiration either natural or artificial in animals under experiment are seen first in the change from well filled to empty jugulars, i.e., decreasing venous return, long before arterial pressure is impaired.

Pointing in the same direction is the fact that voluntarily forced breathing in man has a markedly depressant action on the circulation. The effect is not upon the heart nor upon the vasomotor mechanism, for the heart is accelerated and arterial pressure is not usually lowered. The circulation is rendered slower; and this, as has been shown in a recent paper from this laboratory, is due to a decrease in the venous return to the heart (8). The cause is clearly the reduction in the CO_2 content (or perhaps fundamentally in the C_H) of the blood, for the disturbance does not occur when the forced breathing is performed into a bag so as to prevent excessive pulmonary ventilation.

Conversely an accumulation of CO_2 in the blood causes an abnormally high venous pressure, a dilatation of veins and an exaggeration of the volume of the venous return out of all proportion to the effect on arterial pressure. One of us has had a considerable and unique experience bearing on this matter in connection with tests of the so-called self-contained oxygen mine rescue apparatus (9). In men wearing such apparatus and walking at three or four miles an hour the differences in the effect of insufficient oxygen without accumulation of CO_2 and the effect of large accumulation of CO_2 (3 to 7 per cent) with ample oxygen are very striking. Under the low oxygen the effects are manifested as a grey cyanosis and fainting. Under high CO_2, with ample oxygen, the color of the skin is good, there is intense throbbing headache, the world turns black before the eyes, the gait becomes staggering but the legs do not usually give way. The most striking symptoms however are the indications of greatly elevated venous pressure. The top of the venous column may be at the level of the face or even higher. The superficial veins of the neck and face are enormously distended, both by this pressure and by the relaxation of their own walls. The picture is in this respect like that in the CO_2 acidosis of some patients with renal disease in whom so far as we can judge the heart is not sufficiently affected to explain the venous congestion.

Haldane (10) independently notes (in gassed soldiers) grey cyanosis without rise of venous pressure from oxygen want without excess of CO_2, and "full blue cyanosis and venous engorgement" when excess of CO_2 is added. He does not mention venous engorgement with bright pink lips from high CO_2 and ample oxygen. (Doctor Haldane himself was the first subject in whom I saw this phenom-

enon (at Guy's Hospital, London, in 1913). The significance of the observation did not occur to me until long afterward when I had seen it many times in other subjects.—Y. H.)

To the factor in the circulation thus indicated the term "veno-pressor mechanism" has been applied in previous papers from this laboratory (5), (11). We would suggest that it consists essentially in the chemical influence, either directly or through a local nervous mechanism (e.g., unineuronic reflexes), upon the caliber of the capillaries and especially of the small efferent vessels, the venules, exerted by the greater or less venosity of the blood in and flowing from the organs,—especially the skeletal muscles. The greater the activity of the muscles the more venous this blood. The efferent vessels are thus relaxed and the outflow from the capillaries into the venous system made easier. The volume of the venous return to the heart is increased, the venous pressure is raised and (within certain limits) the efficiency of the heart is increased (11). Thus the two extremes of high venous pressure on the one hand and abnormally low venous pressure on the other are induced respectively, the former by muscular exertion with great CO_2 production and the latter by acapnia and related conditions.

Cannon (12) has recently reported observations on wounded soldiers which suggest that in shock the decrease of venous return may be due to a narrowing of the capillaries and venules so that the red blood cells are accumulated in the periphery. A decrease of flow through the arm and a marked pallor of the skin have been observed here, both after forced breathing and ether hyperpnoea (6), (8).

In these statements we are not denying or questioning the results of a long line of investigators whose work has hitherto been accepted as demonstrating the vasomotor control of the venous return. In fact our own experiments given below show that the vasomotor nervous system exerts a marked influence upon venous pressure. Our view is merely that in addition to this influence there is a mechanism providing a direct peripheral chemical regulation of the venous return to the heart. In support of this view we offer a new and, we believe, crucial observation. Kaya and Starling found that when the lungs of a headless animal are ventilated with air containing a high percentage of CO_2, arterial pressure is not affected (13). We have verified their statement on this point, but we have to add to it a new fact, namely that in such an experiment *venous pressure undergoes a very marked increase.*

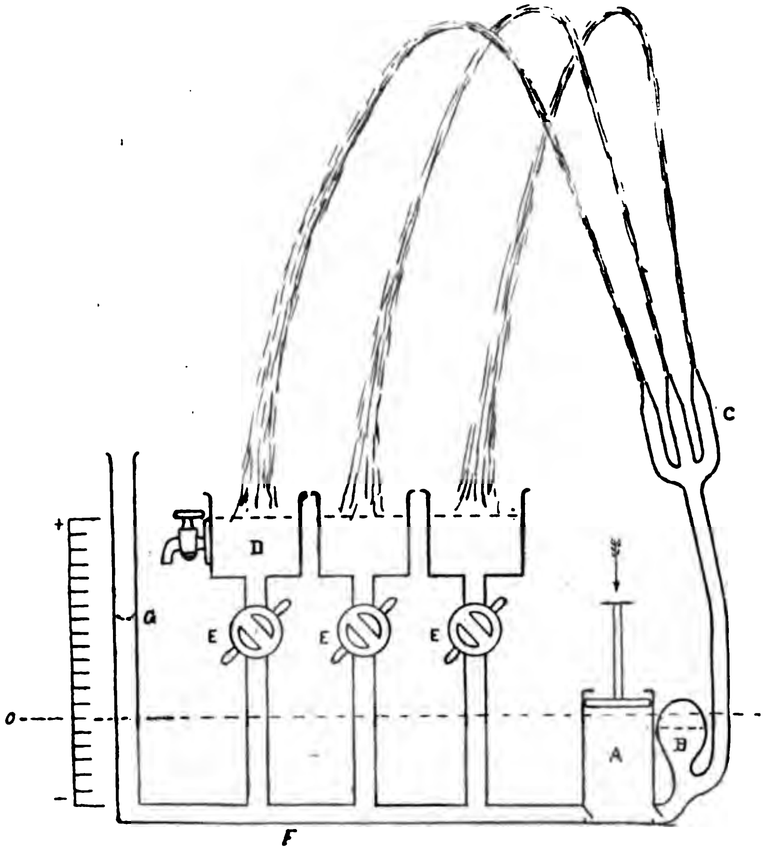

Fig. 1. Diagram to illustrate how a relaxation of venules may be expected to induce a rise of venous pressure. The system shown consists of a pump, *A*, an elastic chamber, *B*, and fine nozzles, *C*, corresponding to the arterioles controlled by the vasomotor nervous system. The jets of fluid after losing a part of the energy imparted by the pump fall into the reservoirs, *D*, representing the capillaries of the tissues and organs. Close below these reservoirs, on the tubes leading back to the pump, are cocks, *E*, which if partly closed will diminish the (venous) return to the pump and cause a lowered (venous) pressure in *F*. If the cocks are widely opened the pressure in the (venous) system, *F*, will rise so that the top of the column, *G*, will be nearly level with the surface of the fluid in the reservoirs. If the cocks are partially closed the column, *G*, will be lowered, and the efficiency of the pump correspondingly decreased.

We hasten to add also that we are not claiming acapnia as the only cause of capillary stagnation and failure of venous return nor hypercapnia as necessarily the sole cause of high venous pressure. Doubtless toxins or cold or inflammation may cause low venous pressure. Facts to be reported in a later paper suggest the identity or close connection of the veno-pressor mechanism with that neuro-vascular element in inflammation upon which such investigations as those of Bayliss (14) and Bruce (15) have begun to throw light.

Probably the tonus of the intestines, which is much affected by the venosity of the blood (6), (16) plays a part also in the rise of venous pressure during muscular exertion. The liberation of stored corpuscles (17), and possibly also osmotic forces, may participate.

It may be asked, how can a dilatation of venules cause a rise of venous pressure? In reply we would point out, (1) That the capacity of the capillaries, i. e., the volume of blood in them, is much greater than that of the venous system. (2) The pressure in the capillaries is much higher than that in the veins,—about one-third of aortic pressure according to v. Kries, as confirmed by Lombard (42). Cotton, Slade and Lewis (40) find the capillaries capable of exerting a constrictive force of at least 30 mm. mercury. (3) When a resistance between two parts of a moving hydraulic system is reduced the difference in pressure between the two parts is correspondingly lessened. In other words, a dilatation of venules should tend to lower capillary pressure and raise venous pressure by amounts inversely proportional to the capacities of the veins and capillaries. These relations are illustrated in figure 1.

Since this paper was sent to press an article by Briscoe (41) has appeared in which measurements of the capillary and venous presssure are given on men by means of the Hooker technique. In eleven normal men the average capillary pressure is found to be 23.5 cm. of water, and in women 22.2 cm. of water or 13.0 and 13.5 higher than venous pressure. In individuals suffering from so-called irritable heart a constriction of venules causing an increase of capillary pressure is described.

LITERATURE

The literature of this subject falls under three main heads: circulatory failure, nervous (i.e., vasomotor) control of the venous return, and the responsiveness of the finer blood vessels to chemical influences.

1. That dealing with the circulatory depression and failure following trauma, toxemia and related conditions, has its beginning in the classic

papers of Goltz (18). In these papers he demonstrated reflex inhibition of the heart and vasomotor nervous mechanism. He thus established the nature of syncope and his explanation stands, probably forever, unshaken. But as regards the circulatory depression of shock the greater part of all the immense literature which has accumulated since Goltz consists merely in continually repeated attempts to make the same explanation apply; and these attempts have been, year after year and investigator after investigator, more and more complete and indisputable failures.

The first to recognize this was Porter (39).

Seelig and Lyon (19), Henderson (5), Mann (20) and others have shown by direct evidence that the explanation based on vasomotor failure is inadequate. Seelig and Lyon, Malcolm (21), and Henderson have emphasized the fact that even when the circulation is failing the arteries are constricted,—just as Wiggers (7) showed in hemorrhage, and that the failure cannot therefore be a vaso-relaxation.

Out of all this mass of experiments and argumentation one fact (first recognized in this laboratory and supported initially against great opposition) seems to have won general acceptance, namely that, while the circulatory depression of syncope consists mainly in a decrease of peripheral resistance in the arteries, *that of shock on the contrary lies almost wholly in decrease of the venous return.* In the one the factor is vasomotor, in the other veno-pressor (12).

2. That vasomotor activity may increase the venous return to the heart and raise venous pressure has been demonstrated by the investigations of Mall (22), Bayliss and Starling (23), Burton-Opitz (24), Plumier (25), Stolnikow (26), Roy and Brown (27) and others, and most clearly of all by those of Carl Tigerstedt (28). We have repeated many of the experiments of these authors ourselves with essentially the results which they obtained; but on the basis of this evidence we are inclined to doubt whether even the most vigorous exertion of the nervous control of the vasomotor mechanism can induce directly the relatively enormous increase of venous flow requisite to maintain and even raise venous pressure when the heart is pumping blood out of the venous system at the rate it does during utmost physical exertion. The usual supposition is that, as Hooker (29) expresses it,

> A vasoconstriction occurs in the great splanchnic area, including the portal vein, which shunts the blood to the active muscles. There is as a consequence a venous plethora which expresses itself as a rise of venous pressure which continues through the period of activity.

Or as Starling (30) expresses it,

> If a man starts to run, his muscular movements pump more blood into the heart, so increasing the venous filling, while the central nervous system, by contracting the arteries of the abdomen, increases the peripheral resistance, raises the arterial pressure, and forces all the available blood through the active muscles.

We do not doubt the qualitative correctness of these statements although we do question whether they tell the whole story. The chemical influence upon the blood vessels exerted by the altered blood gases during exercise must, we think, be taken into account as an accessory to the muscular movements and nervous vasoconstrictions and dilatations in respect to the rise of venous pressure.

3. The responsiveness of the blood vessels to direct chemical stimulation was observed first in capillaries by Severini (31).

Gaskell (32) contributed well known and important experiments and imputed great importance to the fact that the chemical changes, especially acid production, going on in an organ during activity may directly bring about a dilatation of the blood vessels of this organ and so, without the intervention of the nervous system, regulate its own blood supply according to its own needs. Gaskell thought that in order to be of any effect upon the volume of blood flow into and through an organ this dilatation must occur in the smaller arteries and not merely in the capillaries. He seems not to have considered the, as it seems to us, even more probable dilatation of the small veins and the more ready outflow into the venous system under the influence of the chemical products of activity.

Bayliss (33) demonstrated that under constant pressure an increase of flow through an organ occurs when CO_2 or other acid is added to a perfusion fluid. He recognized that any acid stronger than carbonic merely liberates CO_2 from carbonates and is itself neutralized. Thus the effects of all acids are brought about through CO_2, although in last analysis the stimulus may depend on C_H.

Hooker (29), Schwarz and Lemberger (34) and v. Anrep (35) have contributed experiments showing with entire unanimity and conclusiveness the relaxing effect of CO_2 and other acids in high dilution on blood vessels while Adler (36) has shown this effect and with special distinctness the contracting effects of dilute alkali. The details of the observations of Adler and previously of Natus (37) in related lines are especially interesting.

Hooker's work was done in part to test the idea of a veno-pressor mechanism as suggested by Henderson. Hooker rejects the idea and Bayliss likewise sees little to support it. Hooker appears to have misunderstood Henderson as claiming that an increase of acidity constricts veins. The contention of the latter was, on the contrary, that a high content of CO_2 in the blood and a high venous pressure are concomitants, and that low CO_2 induces low venous pressure. If the conception of the circulation expressed in figure 1 is at all correct, it is a mechanically erroneous idea that to raise venous pressure there must be a constriction of veins, and that dilatation of veins causes lower venous pressure. Hooker and Bayliss seem to read "veno-pressor" as synonymous with "veno-constrictor." On the contrary, as we conceive the hydraulic relations a widening of the caliber of the venules, allowing readier outflow from the tissue reservoirs, should increase venous pressure; and a constriction of venules should decrease the venous return to the heart, and lower venous pressure by damming the blood in the capillaries (see fig. 1).

None of the investigators in this field seem to have suggested that the chemical influence of tissue metabolism must be far more completely exerted upon venules than upon arterioles. Gaskell thought that arterioles may be affected through the lymph. But the inner wall of these vessels is bathed by the fresh arterial blood in which the CO_2 content varies comparatively little. Furthermore in normal life the chief control of the arteries is not chemical but nervous and is exerted from the central nervous system. The walls of the venules on the contrary are exposed both from within and without to the influence of metabolites and especially to the full and widely varying venous tension of CO_2, H_2CO_3, and C_H.

The experiments reported below are a few abbreviated illustrations taken from a very large mass of work. They were all performed and reported in outline eight or ten years ago (2). Full publication has been delayed until now in the hope that we should find some form of presentation which would bring the facts of venous pressure under the prevalent vasomotor conceptions.

EXPERIMENTAL

The general plan of our research was to do two sets of experiments for comparison. In one, series *A*, the nervous vasomotor mechanism was excited or depressed by the classic methods—section and stimulation of the spinal cord, stimulation of the splanchnic nerves, stimulation of an afferent nerve and intravenous injection of epinephrin. In the other, series B, as nearly as possible only the purely peripheral chemical factor in the regulation of the circulation was brought into activity by adding CO_2 to the oxygen with which the lungs were insufflated in a decapitated animal. In both series the arterial pressure was taken as the index of the activity of the vasomotor nerve endings. In both it is the amount of the effect obtained upon venous pressure which is the point of interest.

To make the line of thought clear, let us suppose that it could be shown that increased activity of the nervous mechanism (e.g., by epinephrin or splanchnic stimulation) caused a great rise of arterial pressure but no change of venous pressure. Suppose again that increase of CO_2 in the blood and tissues caused a marked rise of venous pressure accompanied by no change of arterial pressure. Then we might fairly differentiate two mechanisms; the one nervous-arterial, the other chemical-venous.

Actually of course the various parts of the circulation are too closely interdependent for such experimental results to be obtainable or even conceivable. Even if the nervous system had no direct control over veins, arterial changes must indirectly influence venous pressure, and even if arterioles were wholly insensitive to chemical influences, venous changes must exert some indirect effect upon arterial pressure. As both of these conditional clauses are contrary to fact the problem of experimental differentiation of the nervous-arterial and chemical-venous controls is one of extreme technical difficulty.

Logically all possibility of the participation in venous pressure changes by alterations of cardiac activity or by stimulation or depression of nerve centers direct or reflex should be experimentally excluded. Such rigor of demonstration appears however to be only partially obtainable.

Any cardio-inhibitory action raises venous pressure by decreasing the volume pumped out of the venous system and by slowing the general blood stream (25). The blood gases strongly influence the cardio-inhibitory center; therefore in most experiments both vagi were cut.

During ordinary asphyxia there is an enormous rise both of arterial and venous pressure even after section of the vagi. If the peripheral effects of CO_2 contribute to this rise, the veno-pressor element is obscured by the powerful central vasomotor influence of asphyxia (38). For demonstration of the veno-pressor factor it is essential that the well-known and powerful effect of the asphyxial chemical substances and deficiencies upon the vasomotor center in the medulla should be excluded. Therefore (except in certain cases) the spinal cord was exposed and cut just below the occiput in dogs, while in the cats used the head was removed by the method of Sherrington.

When the stump of the spinal cord is stimulated electrically to determine the effects of electrical excitation of the vasomotor mechanism upon the circulation all the skeletal muscles also are thrown into contraction and this contributes to the rise of pressure by an indefinable amount. Therefore the animals in which this experiment was tried were always curarized.

When the cord is electrically stimulated or when epinephrin (Parke, Davis & Company adrenalin) is introduced into a vein it is necessary to distinguish between the effect upon venous pressure induced directly and effects indirectly resulting from the slowing or overstrain of the heart. Vagus section and care as to dosage are the only, yet not wholly satisfactory, means of covering this point.

Fortunately for the purpose of our research the results of the first series of experiments indicate that the nervous control of the circulation is chiefly on the arterial side, while the results of the second series demonstrate that the peripheral chemical control is powerful in its influence upon venous pressure and scarcely perceptible in its relation to arterial pressure.

Series A. These experiments are controls showing the maximum extent of the vasomotor nervous influence upon venous pressure. They show that *a*, abolition of the influence of the vasomotor center in the medulla by section of the spinal cord causes no fall (but sometimes indirectly a rise) in venous pressure; *b*, stimulation of the stump of the spinal cord sufficient to cause a marked rise of arterial pressure has only a slight and apparently mainly indirect effect in raising venous pressure when skeletal muscular movements are excluded by means of curare; *c*, reflex rise of venous pressure on stimulation of an afferent nerve is not comparable in amount to the arterial rise; *d*, stimulation of the splanchnic nerves induces a rise of both arterial and venous pressure. But in none of these conditions do the changes in venous pressure exceed 35 mm. saline and they are seldom so much, although the alteration of vascular tonus as judged by changes of arterial pressure, was in many cases intense,—facts which contrast strikingly with the results of chemical stimulation to be shown later (series *B*).

Methods. When dogs were used the technique was usually as follows: morphine subcutaneously (0.010 to 0.015 gram per kilo body weight); half an hour later a little chloroform, then ether. Vagi cut. Arterial pressure recorded by a manometer connected with the carotid artery. Venous pressure taken in the central end of the jugular or femoral vein by the method described by Henderson and Barringer (11). Great care was taken, and is necessary, to insure avoiding any obstruction in the vessel. Even a full bladder obstructs the femoral return. Only the results of quick inflows were recorded (cf. loc. cit. (11)). Measurements of venous pressure are in millimeters of saline solution. Zero pressure was taken as the level of the junction of the vessel into which the special venous cannula manometer was inserted, and its proximal vein, the subclavian or abdominal vena cava. The protocols state when curare was used. After two or three concordant measurements of arterial and venous pressure the spinal cord was cut between the atlas and occiput and the effects upon arterial and venous pressures were noted. When the cord was stimulated electrically the electrodes were inserted into the vertebral canal on each side of the cord to a distance of a few millimeters below the point of section. Artificial respiration, unless otherwise indicated, was maintained by means of a pump run by an electric motor, or by intratracheal insufflation of oxygen from a cylinder of compressed gas through a wash bottle.

In some experiments the vena cava was partly occluded as a device to exclude cardiac effects upon venous pressure and confine the evidence to peripheral changes.

In the experiments in which the splanchnics were stimulated the spinal cord and vagi were not cut.

When cats were the subjects of the experiment they were first lightly etherized, the vagi cut and the cord severed just below the occiput, or else the entire head was removed by the method of Sherrington.

Experiment A 1. Bull dog; 5 kilos. Venous pressure from jugular vein. Insufflation.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before section of spinal cord	110	30
20 seconds after section	80	50
1 minute later	60	50
2 minutes later	60	50

Result: On section of the cord venous pressure, instead of falling, rose slightly, owing probably either to the passage of a greater volume of blood through the relaxed arterioles into the venous system, or to less efficient heart action.

Experiment A 2. Dog; 9.5 kilos. Venous pressure from femoral. Insufflation. The vena cava was partly compressed by a ligature placed about it just below the diaphragm, not sufficient to occlude it entirely but merely so as to cause and maintain a high venous pressure distally. It was thought that under these conditions any change in the tonus of the veins would be made more evident.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
6 minutes before section of spinal cord	120	240
Immediately before section	115	240
1 minute after section	90	240
4 minutes after section	80	240

Result: With a slight obstruction (not occlusion) of the vena cava the femoral venous pressure was not affected by spinal section although arterial pressure fell 35 mm.

Experiment A3. Bull dog; 9 kilos. Venous pressure from femoral vein (unobstructed). Insufflation. The animal was fully curarized.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before section of cord	160	60
3 minutes after section	80	75
4 minutes after section	70	60
5 minutes after section	60	50

The spinal cord was then stimulated electrically for 2 minutes, i.e., through 8th minute

6 minutes after section	80	60
7 minutes after section	90	60
8 minutes after section	100	60
12 minutes after section	55	55

Stimulation of cord repeated with stronger current and continued for 6 minutes (through 17th minute)

13 minutes after section	95	65
15 minutes after section	125	75
17 minutes after section	150	60
35 minutes after section	70	60

Result: On section of the cord arterial pressure fell one-half while venous pressure rose slightly, then fell slightly. On moderate stimulation of the cord there was marked rise of arterial, but only a slight rise of venous pressure. On strong stimulation arterial pressure was raised from 55 to 150 while venous pressure rose only from 55 to 75, and failed to maintain even this elevation to the end of the stimulation period.

Experiment A4. Bull dog; 13 kilos. Venous pressure from jugular. Artificial respiration after section of cord.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury.	*mm. saline.*
Before section of cord	120	45
15 seconds after section of cord	60	40
30 seconds after section of cord	40	40
40 seconds after section of cord	35	45
1 minute after section of cord	35	40
7 minutes after section of cord	35	40

Cord stimulated electrically for 1 minute

9 minutes after section of cord	120	45
11 minutes after section of cord	60	30

Cord stimulated for 2 minutes

13 minutes after section of cord	100	45
14 minutes after section of cord	100	40
15 minutes after section of cord	80	30
16 minutes after section of cord	65	30
34 minutes after section of cord	30	35

Cord stimulated for 5 minutes, (through 40th) curarization had begun to wear off

36 minutes after section of cord	105	70
37 minutes after section of cord	100	65
38 minutes after section of cord	100	60
39 minutes after section of cord	100	50
40 minutes after section of cord	100	40
41 minutes after section of cord	80	35
42 minutes after section of cord	70	30

Result: On section of spinal cord arterial pressure fell from 120 to 35 with a barely perceptible change of venous pressure. Stimulation raised arterial pressure greatly, venous pressure only slightly. After the curare had begun to wear off and slight muscular movements were induced by spinal stimulation, a marked but not a lasting effect on venous pressure was induced.

Experiment A5. Dog; 9 kilos. Venous pressure from jugular. Insufflation. Animal curarized. Spinal cord cut. Left sciatic nerve exposed and cut. Central end of nerve stimulated electrically.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	70	25
Stimulation of sciatic for 6 minutes		
1st minute	80	30
2d minute	90	30
3d minute	100	30
4th minute	110	30
5th minute	120	30
6th minute	130	30

Result: After section of the spinal cord stimulation of the central end of the sciatic nerve caused arterial pressure to rise gradually from 70 to 130 mm. without a corresponding effect on venous pressure.

Experiment A6. Dog; 10 kilos. Morphin. Ether. Artificial respiration. Stimulation of right splanchnic in thorax.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	120	30
Splanchnic stimulation	150	50
After stimulation	110	20

Result: Stimulation of the splanchnic caused a rise of both arterial and venous pressures.

Experiment A7. Dog; 12 kilos. Morphin, ether, artificial respiration. Right splanchnic nerve stimulated in thorax.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before stimulation	120	45
Splanchnic stimulation	150	55
Stimulation stopped	130	45
Stronger stimulation	160	55
Stimulation stopped	130	45

Result: Stimulation of the splanchnic nerve caused a rise of both arterial and venous pressure.

Experiment A8. Dog; 11.5 kilos. Morphin, ether. Natural breathing of air under a sufficient positive pressure to keep the lungs distended after the thorax was opened. A cardiometer was placed upon the ventricles of the heart and the volume curve recorded. From the heart rate and amplitude of beat the circulation rate has been calculated. The right splanchnic was cut and stimulated.

	HEART RATE BEATS PER MINUTE	CIRCULATION RATE	ARTERIAL PRESSURE	VENOUS PRESSURE
		cc. per minute	*mm. mercury*	*mm. saline*
Before section of splanchnic	90	1,980	140	70
After section of splanchnic	100	1,300	95	45
During stimulation of splanchnic	100	1,600	130	50
During stronger stimulation	85	1,745	165	60
After stopping stimulation	65	1,495	110	50

Result: Section of the right splanchnic caused a fall of both arterial and venous pressure, a less efficient diastolic filling of the heart and a consequent decrease of circulation rate. Stimulation of the splanchnic raised both pressures and increased the venous return as evidenced by augmentation of the circulation rate. For graphic records from this animal see Henderson and Barringer, op. cit. p. 360.

Experiment A9. Cat, decapitated. Insufflation. Quick injection of adrenalin, complete in 10 seconds.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	95	50
30 seconds after	140	70
2 minutes after	80	50

Result: The arterial pressure rose 45 mm. and venous 20 mm.

Experiment A10. Cat, decapitated. Insufflation. Slow injection of adrenalin during 2 minutes.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	20	5
20 seconds after starting injection	150	40
1 minute after starting injection	150	30
2 minutes after starting injection	150	10
2½ minutes after starting injection	140	5
5 minutes after starting injection	30	5

Result: Although venous pressure rose at first it fell again even during the injection nearly to its initial level, indicating that the vasomotor influence upon venous pressure is not direct but is due to redistribution of blood. As soon as this redistribution is established venous pressure falls again even while a high arterial pressure is maintained.

Experiment A11. Cat, decapitated. Insufflation. Slow injection of adrenalin, during 4.5 minutes.

	ARTERIAL PRESSURE	VENOUS PRESSURE
	mm. mercury	*mm. saline*
Before injection	40	0
30 seconds after starting injection	150	20
1 minute after	150	20
2 minutes after	140	10
3 minutes after	130	5
4 minutes after	120	5
5 minutes after	100	0

Result: Although venous pressure rose at first it fell again even during the injection nearly to its initial level.

Series B. These experiments were designed to test the peripheral chemical vascular control, i.e., the veno-pressor mechanism. They were performed upon cats after decapitation, or section of vagi and spinal cord. The pulmonary ventilation was maintained by intratracheal insufflation with a jet of oxygen.

When the jet was enriched with 15 or 20 per cent of CO_2, venous pressure began after a few seconds to rise and continued to do so until levels of 60 or 70 mm. or even more were attained. When the CO_2 was shut off and the lungs ventilated rapidly with oxygen alone, venous pressure fell again gradually to its original level.

These changes of venous pressure are about 100 per cent greater than those obtained in the previous series of experiments. Their significance is clear in view of the fact that, while in the previous series arterial pressure was always greatly affected, in the effects of CO_2 arterial changes are minimal or absent. The only effect upon the arterial pressure curve was an increased amplitude of pulsation.

Venous pressure was measured from the jugular in all cases and from the femoral also in a few experiments in which the vena cava inferior was partially occluded.

Experiment B1. Cat, beheaded. Insufflation with oxygen, then oxygen plus 15 per cent CO_2, and finally oxygen alone.

	TIME	ARTERIAL PRESSURE	VENOUS PRESSURE IN JUGULAR
	minutes	*mm. mercury*	*mm. saline*
Oxygen alone	0	60	0
Oxygen + CO_2	1	60	5
Oxygen + CO_2	2	62	10
Oxygen + CO_2	2½	62	25
Oxygen + CO_2	3	64	35
Oxygen + CO_2	3½	64	40
Oxygen + CO_2	4	66	50
Oxygen + CO_2	4½	68	60
Oxygen + CO_2	5	70	70
Oxygen alone	5½	70	60
Oxygen alone	6	70	50
Oxygen alone	7	70	45
Oxygen alone	8	70	30
Oxygen alone	9	68	15
Oxygen alone	9½	68	10
Oxygen alone	10	66	5
Oxygen alone	11	66	0

Result: Under the influence of CO_2 venous pressure rose progressively during 5 minutes to a height of 70 mm. As the excess of CO_2 was ventilated out of the animal venous pressure fell again. The simultaneous changes of arterial pressure were 10 mm. upward and 4 mm. downward. The pressure record showed an increased amplitude of pulsation.

Experiment B2. Cat, beheaded. Insufflation with oxygen, then oxygen plus 15 per cent of CO_2, and finally oxygen alone.

	TIME	ARTERIAL PRESSURE	VENOUS PRESSURE
	minutes	*mm. mercury*	*mm. saline*
Oxygen alone	0	50	0
Oxygen + CO_2	1	50	0
Oxygen + CO_2	7	52	10
Oxygen + CO_2	16	58	30
Oxygen + CO_2	19	58	40
Oxygen + CO_2	20	60	45
Oxygen alone	22	60	60
Oxygen alone	23	60	50
Oxygen alone	24	60	45
Oxygen alone	25	60	35
Oxygen alone	26	60	25
Oxygen alone	27	55	10
Oxygen alone	28	55	0

Result: Under CO_2 venous pressure rose during 22 minutes from 0 to 60 mm. while arterial pressure rose only from 50 to 60. The amplitude of the pulsations in the arterial pressure record was markedly increased. Later during 6 minutes under oxygen alone venous pressure fell to its original level and arterial pressure sank slightly.

Experiment B3. Cat, vagi cut and spinal cord severed below occiput with dull chisel but head not removed. Insufflation with oxygen, then oxygen plus 20 per cent of CO_2, and finally oxygen alone. The vena cava inferior was partially (not completely) occluded and the pressure was determined in both the jugular and femoral veins.

	TIME	ARTERIAL	PRESSURES JUGULAR VEIN	FEMORAL VEIN
	minutes	*mm. mercury*	*mm. saline*	*mm. saline*
Oxygen alone	0	60	30	50
Oxygen + CO_2	1	70	20	65
Oxygen + CO_2	4	70	25	80
Oxygen + CO_2	5	70	35	85
Oxygen + CO_2	6	70	35	90
Oxygen alone	8	70	35	70
Oxygen alone	10	70	35	65
Oxygen alone	11	65	30	60
Oxygen alone	12	65	20	50
Oxygen alone	13	60	20	45
Oxygen alone	14	60	20	30

Result: The effects of CO_2 in this case were similar to those in the two preceding experiments but the fact that increased amplitude in the arterial pressure record occurred even with the vena cava damped suggests that this phenomenon is not due to increased filling of the heart, i.e., larger systolic discharges, but to more forcible contractions.

CONCLUSIONS

The general significance of the results of these two series of experiments appears to us to be as follows:

1. Procedures which strongly influence vasomotor innervation, e.g., spinal section, spinal stimulation, stimulation of an afferent nerve, splanchnic stimulation and intravenous injection of epinephrin, cause on the whole decidedly greater alterations of arterial than of venous pressure, and the alterations of venous pressure are often only momentary and therefore largely indirect and secondary to redistribution of blood.
2. In the beheaded cat increase of CO_2 in the blood (with ample oxygen), which has little or no effect upon arterial pressure other than an increased amplitude of pulse, causes an enormous rise of venous pressure. The pressure develops gradually as the CO_2 accumulates in the tissues and falls again gradually as the excess of CO_2 is ventilated out of the body.

3. These facts, taken with those quoted in the introductory part of this paper, indicate the existence of a veno-pressor mechanism distinct from the vasomotor nervous regulation and consisting in a peripheral chemical control, largely through variations in the CO_2 content of the venous blood, over the venous pressure and the volume of the venous return.

4. As venous pressure and the volume of the venous return are essential elements in the diastolic filling of the heart, and thus are factors in determining the volume of blood circulated in unit time, it appears probable that the veno-pressor mechanism may play a part in the increased circulation during muscular exertion. It thus assists in coördinating the volume of the blood stream with the energy expenditure and gaseous metabolism of the tissues.

5. As it is now generally admitted that in shock it is the decreased venous return which is the cause of the fall of arterial pressure, the relation of CO_2 to the veno-pressor mechanism affords an explanation of the mode by which acapnia induces circulatory failure.

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XII. THE INFLUENCE OF DRUGS ON INTESTINAL RHYTHMICITY

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It has been shown that, roughly speaking, the rate of rhythmic contraction in the small intestine varies inversely as the distance from the pylorus (1). The evidence in favor of the muscular origin of this rhythm seems now to be overwhelming. The theory of a neurogenous origin depended almost entirely upon the work of Magnus, which has recently been repeated and overthrown by Gunn and Underhill (2). Using a better technic, they obtained plexus-free strips of intestinal muscle that would contract rhythmically in Locke's solution. More recently Mr. Taylor and I (3) and Miss Starkweather and I (4) have presented evidence in support of the view that the local differences in rhythmicity are due to differences in the metabolism of the muscle in the different regions. This present work was undertaken in the hope that further light might be thrown upon this problem by a study of the ways in which various drugs influence the rate of contraction in the different regions.

TECHNIC

Excised segments of rabbit's intestine have been used. The animals were killed by a blow on the head and the abdomens opened immediately. Segments were cut from the first portion of the duodenum, from the upper jejunum, from the upper ileum, from the lower ileum and from the colon where it parallels the first portion of the duodenum. These pieces were washed out and kept in iced Locke's solution. Smaller segments, 2 to 3 cm. long, were cut as needed. These were attached to light heart-levers and suspended in a beaker containing 400 cc. of aerated Locke's solution kept at 38°C. When testing valuable drugs this large amount of fluid may seem wasteful but the results are much better than those obtained with small amounts. The segments beat more regularly, possibly on account of the greater dilution of their metabolites; and less care need be taken to avoid changes in temperature when adding drugs. To insure prompt mixture the drugs were all added in liquid form. By directing the air-jet horizontally, a certain amount of circulation was kept up in the fluid. Most of the work was done with new segments which had not been tested previously with any drug. Ordinarily the segments that had been tested and washed were used for preliminary work in finding the dosage of the various drugs which would definitely influence the muscle but not paralyze it. After that a few crucial experiments would be done with new pieces.

Of the seventy-five drugs studied, a number are used commonly in medicine as laxatives and emetics, several are nerve depressants and anesthetics, some diminish or increase oxidation while others are poisons which attack various parts of the protein molecule. They have been arranged alphabetically for the sake of convenience. They may be divided into three classes: those which increased the rate, those which slowed it and those which had no definite effect with the doses used.

When possible, the rate was counted after the segments had been exposed to the drug for from ten to fifteen minutes. Sometimes this interval had to be shorter, as when the contractions ceased or became irregular. No attempt was made to estimate percentages of increase or decrease in the rates of the colonic segments after it was seen that they contracted too irregularly for accurate work. It is interesting, as showing the great difference between the muscle in small and large intestine that except in the case of a few drugs which made the colonic rhythm more regular and others which stopped it altogether, those which had pronounced effects on the rates of the small intestine had none on the rate of the large.

Drugs which increased the rate. The most pronounced effects were obtained with calcium chlorid, calcium lactate and benzene. The following tables show data obtained in typical experiments. In all these tables D stands for duodenum, J for jejunum, M for middle and I for ileum.

	WITH CALCIUM CHLORID									WITH CALCIUM LACTATE			WITH BENZENE		
	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage	Before	After	Percentage
D............	16.0	16.0	100	15.5	17.0	109	14.0	17.5	125	15.0	16.0	107	17.2	19.5	113
J............	14.3	14.5	101	14.5	17.8	123	12.0	16.8	140	13.0	15.5	119	15.0	19.2	128
M............	12.7	14.2	112	14.0	17.5	125	11.0	16.2	147	12.5	16.5	132	14.5	18.0	134
I............	12.0	14.0	117	12.0	16.0	133	9.5	14.3	150	12.5	16.0	128	10.5	14.5	138

A similar graded effect was observed in some of the experiments with sodium and potassium hydrate.

	WITH SODIUM HYDRATE			WITH POTASSIUM HYDRATE		
	Before	After	Percentage	Before	After	Percentage
D.	15.0	17.0	113	12.5	12.0	96
J.	13.0	15.0	115	12.0	11.5	96
M.	11.0	15.0	136	9.0	10.3	114
I.	10.8	14.5	134	8.5	9.3	109

More often, however, the percentage of increase was about the same or else graded irregularly. Nicotin occasionally produced a graded increase in rate, as will be seen from the following figures.

	WITH NICOTIN					
	Before	After	Percentage	Before	After	Percentage
D.	15.7	15.8	101	14.3	14.3	100
J.	14.0	14.3	102	13.0	13.5	104
M.	14.0	14.5	103	12.8	12.5	98
I.	12.3	14.0	113	9.3	10.0	107

Ordinarily the results were more irregular. Ammonia gave one graded result among several atypical ones.

	WITH AMMONIA		
	Before	After	Percentage
D.	15.6	16.0	102
J.	10.8	12.0	111
M.	10.2	11.5	113
I.	8.2	11.6	141

A pronounced ungraded increase was observed several times with mercuric chlorid. Slight irregular increases were obtained at times with adrenalin, acetone, chloroform and ether. Ordinarily no changes in rate were observed with these drugs.

The striking thing, then, in a number of these experiments was the peculiar gradation in the percentage of increase. The possible significance of this finding will be taken up later in the discussion.

Drugs which slowed the rate. These may be divided first into two groups: those which had pronounced effects and those which had only slight effects.

Pronounced effects

Aloin
Alum $AlK(SO_4)_2 12H_2O$
Antimonyl potassium tartrate
Carbon dioxid
Cascara
Chloral hydrate
Digitalis
Ergot
Formaldehyd
Hydrochloric acid
Ipecac
Jalap
Phenyl hydrazin
Potassium cyanid
Quinin bisulfate
Quinin hydrochlorid
Quinin urea hydrochlorid
Senna
Sodium citrate
Sodium nitrite
Sodium salicylate
Zinc sulfate

Slight effects

Alcohol (ethylic)
Anilin oil
Apocodein hydrochlorid
Apomorphin
Barium chlorid
Beta eucain
Chloretone
Copper sulfate
Digitalin
Glucose
Hydrogen peroxid
Lead acetate
Magnesium chlorid
Magnesium sulfate
Oxalic acid
Phenol
Pilocarpin
Potassium bromid
Potassium iodid
Potassium permanganate
Sodium fluorid
Sodium phosphate (Na_2HPO_4)
Sodium potassium tartrate
Sodium sulfate

Thirteen of these substances will be discussed first as they all tended to slow the segments in a particular way. All but alcohol are from the group of strong depressants. They are:

Alcohol
Alum
Antimonyl potassium tartrate
Carbon dioxid
Chloral hydrate
Digitalis
Ergot
Formaldehyd
Potassium cyanid
Quinin bisulfate
Quinin hydrochlorid
Quinin urea hydrochlorid
Sodium citrate

Following are some typical protocols:

	DIGITALIS			CHLORAL HYDRATE		
	Before	After	Percentage	Before	After	Percentage
D.............	12.4	12.5	100	16.8	11.0	66
J.............	11.5	10.0	87	11.4	6.0	53
M.............	8.7	6.5	74	10.2	5.0	49
I.............	8.2	7.5	91	9.2	5.0	55

	POTASSIUM CYANID					
	Before	After	Percentage	Before	After	Percentage
D	17.0	15.0	88	12.3	12.0	97
J	14.0	12.0	86	10.2	6.0	58
M	12.0	6.5	54	10.5	5.0	47
I	12.0	8.5	71	9.8	4.4	45

	CARBON DIOXID					
	Before	After	Percentage	Before	After	Percentage
D	13.9	11.3	81	14.0	13.2	94
J	13.7	11.0	80	13.5	11.5	85
M	11.7	6.4	55	11.5	8.2	71
I	10.6	5.4	51	10.0	9.0	90

Twenty-four such sets of percentages obtained with different drugs have been charted in figure 1. The ordinates represent percentages and the abscissae the four segments. It is apparent that there was always a gradation from segment D to segment M and then usually a rise to segment I. Occasionally the downward gradient was maintained as far as segment I. The percentage of I never rose as high as that of D.

The other drugs in the group of marked depressants which did not show this type of gradation are, with two exceptions, resinous vegetable extracts: aloin, cascara, ipecac, jalap and senna. With these, and with all but one (alcohol) of the mild depressants in group 2, the rates were depressed either irregularly or about equally.

Substances which did not affect the rate.

Acetone. Occasionally seemed to increase the rate
Adrenalin. Occasionally seemed to increase the rate
Atropin
Bile
Chloroform. Occasionally seemed to increase the rate
Cocain. Occasionally seemed to increase the rate
Codein. Occasionally seemed to decrease the rate
Eserin
Ether. Occasionally seemed to increase the rate
Glycerin
Lithium carbonate
Morphin sulfate
Novocain
Picric acid
Pitu trin
Potassium chlorid
Sodium bicarbonate
Sodium chlorid
Strychnin sulfate
Urea
Urethane.

It is possible that if larger doses had been used some of these drugs might have produced more definite effects. Some of them, such as chloroform and ether, could not be used in larger concentration on account of their comparative insolubility in water.

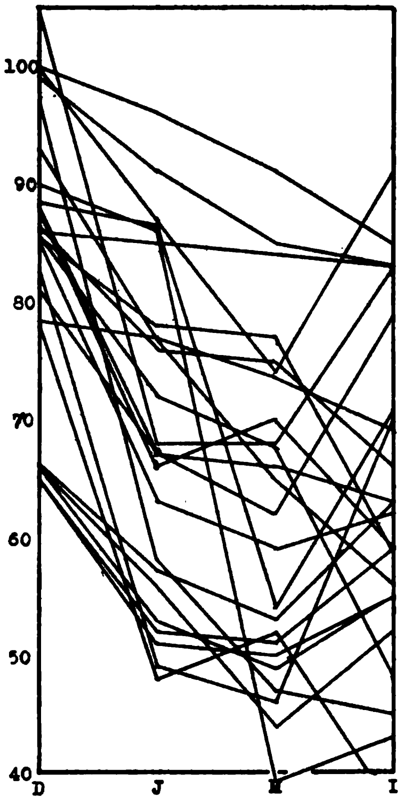

Fig. 1. Shows the gradation in the percentage of slowing in different regions. The ordinates represent percentages of the original rate before the drug was added; the abscissae represent the four regions from which segments were taken.

DISCUSSION

At first sight there seems little in common among the drugs which increased the rate. Ammonia and the hydrates of potassium and sodium may owe their effects to their alkalinity. Sodium bicarbonate, however, had no effect on the rate although it had definite effects on tone and amplitude of contraction. Hydrochloric acid and CO_2 slowed the segments markedly but oxalic acid had little effect on the rate and picric acid had none. To be sure, the toxic effects of the last two acids were so marked that, in the small doses used, the acid factor may have been slight. Calcium salts are known to favor rhythmic activity in the heart muscle. It is strange, however, that they should, at the same time that they increase the rate, markedly decrease the amplitude of contraction. Similarly it is surprising to find that drugs which markedly increase the tone of the muscle, such as barium chlorid, copper sulfate, potassium chlorid, pilocarpin, eserin and pituitrin have, if anything, a depressant effect on the rate of contraction. Again, adrenalin, urethane and atropin, which diminish the tone and amplitude of contraction, do not perceptibly alter the rate.

These observations suggest that there are two distinct phases of the muscular metabolism acted upon: one concerned with the maintenance of tone and amplitude, the other with the rate of rhythmic contraction. Taylor and I (3) have shown that a rise in temperature will affect both together. As is well known, a big rise or drop in tone will generally affect the amplitude of contraction and will extinguish the rhythmic waves.

Nicotin may possibly have stimulated ganglion cells in Auerbach's plexus, although, as I have stated above, it is unlikely that this plexus has anything to do with the rhythm. It should be noted in this connection that adrenalin and atropin which are supposed to act on nerve endings had no effect on the rate. Neither did pituitrin, cocain, novocain, morphin, chloroform, ether or urethane. Pilocarpin, which stimulates nerve endings, had actually a depressant effect on the rate. Some of the drugs whose main effect is a depressant one on nervous tissue did have a slowing effect on the rate. Among these may be mentioned betaeucain, chloretone, phenol, chloral hydrate, magnesium sulfate, potassium bromid, quininurea hydrochlorid and alcohol. It must be remembered, however, that most if not all of these drugs have, besides their marked and well known effects on nerves, a more or less toxic action on muscle and on protoplasm in general. Those who may feel inclined to use these results in arguments for or against the neurogenous origin of the rhythm should be deterred not only by the contradictions pointed out above but also by the many contradictions in recent writings; contradictions which show that our ideas about the pharmacology of nerve endings must be revised. Some of these difficulties in the way of accepting the old ideas are well brought out by Cushny (5). It is not surprising that protoplasmic poisons such as potassium cyanid, formaldehyd, phenylhydrazin, anilin, oxalic acid and potassium permanganate should have slowed the rate. Others, however, such as picric acid, had no effect; while mercuric chlorid and ammonia produced an increase in rate.

The gradations in the percentage of increase and decrease with various drugs are of considerable interest. They suggest a greater stability of the rhythm in the duodenum. The tendency to contract rhythmically is so much stronger in the upper end of the tract that it may, perhaps, overcome a depressant which is powerful enough to slow the ileum (6). The greater increase in the rate of the ileum after the addition of calcium salts suggests that the duodenal rate is already nearly maximal. The fact that some of the drugs produced graded increases or decreases while others had ungraded effects suggests that the two groups act in different ways or on different parts of the contractile protoplasm.

It is of interest that the vegetable cathartics, aloin, cascara, jalap and senna all produced a marked decrease in the rate of contraction. This effect could not be ascribed to the alcohol introduced with them as the amount was too small to have any effect. Another thing which deserves mention is the fact that digitalis had a pronounced slowing effect on the excised intestinal muscle just as it has on the heart (7). That CO_2 has a particular effect on the rate aside from its asphyxiating action is shown by the fact that no slowing appeared even when the segments were allowed to contract for long periods of time without aeration of the fluid.

SUMMARY

Sets of five segments from different parts of the bowel were studied under identical conditions in a beaker of aerated Locke's solution.

Of the seventy-five drugs tested, eight increased the rate, forty-six slowed it and twenty-one had no effect.

Calcium chlorid, calcium lactate, benzene, nicotin, ammonia, sodium hydrate, potassium hydrate and mercuric chlorid increased the rate.

Marked slowing was produced by alum, antimonyl potassium tartrate, carbon dioxid, cascara, chloral hydrate, digitalis, ergot, formaldehyd, hydrochloric acid, ipecac, jalap, phenylhydrazin, potassium cyanid, quinin salts, senna, sodium citrate and sodium nitrite.

With a number of the drugs the rate of the ileal segment was more affected than that of the duodenal segment; and there was a gradation in the percentage of increase or decrease from one end of the bowel to the other. This suggests that the rate in the upper part of the gut is more stable and perhaps more nearly maximal than it is in the ileum.

Drugs which increase or decrease the tone and amplitude of contraction do not necessarily affect the rate. Thus, pilocarpin and barium chlorid which increased the tone, decreased the rate. Calcium salts which increased the rate diminished the amplitude of contraction. This suggests two phases of muscular metabolism acted upon: one concerned with tone and amplitude, the other with the rate.

Digitalis slows the intestinal contractions much as it slows those of the heart.

Excepting those cases in which the colonic rhythm was stopped entirely, it was practically unaffected by the drugs which caused marked changes in the rate of the small intestine.

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XIII. THE MOTOR FUNCTIONS OF THE CECUM

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It has been shown in recent papers that there are gradients of rhythmicity, irritability, latent period, CO_2 production and catalase content in the small intestine; gradients which we believe determine the direction of peristalsis (1). It occurred to us that there might be similar gradients in the muscular wall of the cecum, determining the direction of peristalsis in that organ. Some provision for the maintenance of orderly contractions would seem necessary, particularly in those birds which have ceca as long as the small intestine itself.

While studying a large number of (anesthetized) rabbits with their abdomens opened under salt solution, Alvarez (2) had an opportunity of watching the movements of the cecum. Except in animals with diarrhoea, this organ showed only occasional contractions at long intervals. The waves generally appeared near the apex, ran toward the base and then back again. Such movements ordinarily took place when a peristaltic rush forced material from the ileum through the ileocecal sphincter. Some of this material would apparently go into the cecum and some into the upper colon. Other observers have described similar movements. Meltzer and Auer (3), who watched the cecum through the shaved abdominal wall in rabbits, saw waves about once a minute. Katsch and Borchers (4), who studied the organ through a glass window in the abdominal wall, found it quiet for hours in some animals and more active in others. Elliott and Barclay Smith (5) say that in the guinea pig food from the ileum goes directly into the cecum. It is then passed backwards and forwards for awhile between cecum and colon. By giving the rabbits a definite number of small glass beads, which could be identified at autopsy several days later, they showed that the cecum retains its contents for many days. Swirski (6) showed that it would empty itself entirely after several days of absolute starvation. Basler (7) found that whereas in rats and cats there is almost no churning of the cecal contents, in rabbits and guinea pigs the material is pretty well mixed.

Rhythmicity of excised muscle. We first looked for differences in rhythmicity in strips of muscle excised from five regions along the rabbit's cecum. The animals were killed by a blow on the head and bits of muscle with mucous membrane attached were cut immediately. For the most part longitudinal strips were used. These pieces, about 1 cm. wide and 3 cm. long, were attached to light heart levers and suspended in a beaker containing 400 cc. of aerated Locke's solution at 38°C. All five of the segments beat poorly, less regularly even than

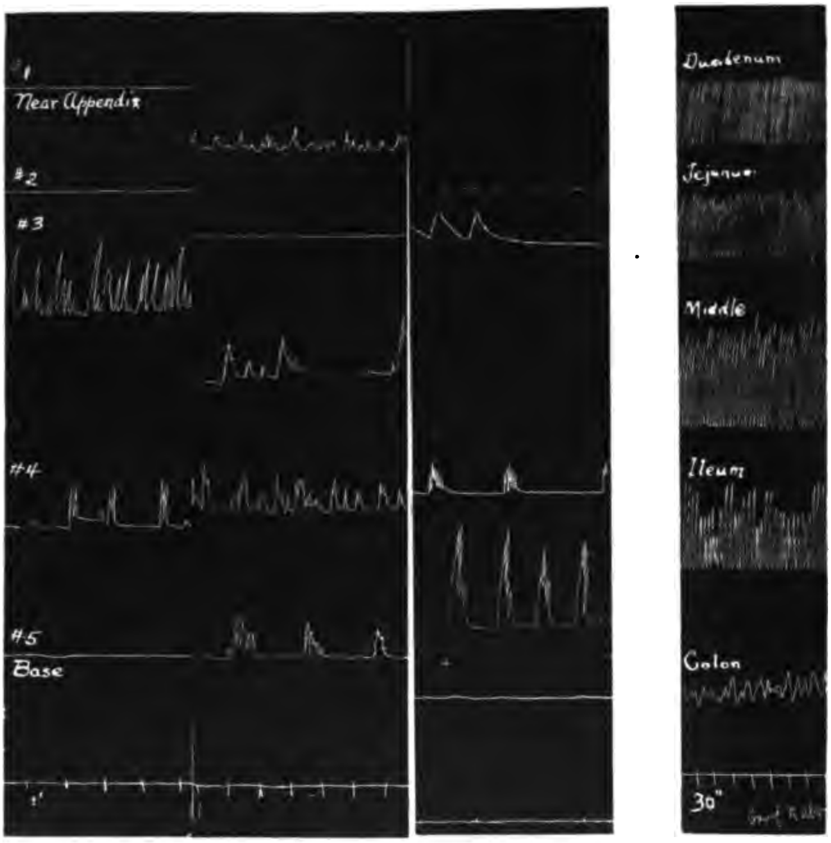

Fig. 1. Tracings from different parts of the rabbit's cecum. On the right, for comparison, tracings from different parts of small intestine and colon.

did the segments of colon which, as we have shown in a previous paper (8), have very little rhythmicity as compared with segments of small intestine. We could not make out much difference in the rhythmicity or in the shape of the contraction curves in different regions. Characteristic tracings are shown in figure 1. At intervals there were tonus waves upon which were superimposed a short series of contractions with a rate of from 8 to 16 per minute. After a while the tonus waves sometimes disappeared, leaving a fairly regular curve with perhaps 5 waves per minute. No difference was observed between the contractions of the longitudinal and of the circular strips. Segments of guinea pig's cecum showed even less tendency to contract rhythmically than did those from the rabbit; and no records of any value were obtained.

A glance at the records from cecum, small intestine and colon in figure 1 will show what a difference there must be in the neuromuscular apparatus in the three regions.

Irritability, latent period and form of contraction curve. Strips of muscle similar to those used in the experiments just described were placed in a warm, moist chamber (temperature 26° to 28°) and stimulated with a strong faradic current. A Harvard inductorium was used with the secondary coil at 0. The source of current was a battery giving 22 amperes at 1.5 volts. Even with this strong current the muscle responded very poorly and after a long latent period. The amplitude was so small and the rise from the base line so gradual that the latent periods could not be measured with any degree of accuracy except perhaps in the case of the strip removed from a point three-quarters of the way from the base of the appendix to the ceco-colonic junction. In the four rabbits studied this segment, number 3, always contracted well with an amplitude, on the tracings, of from 3 to 4 cm. The other segments raised the writing-point only from 0.2 to 1.2 cm. The lever magnified about four times. The following data are only approximately correct and would not be presented were it not that the gradation is so similar in all.

Latent periods

Number 1 (near tip)	0.7	0.6	1.0
Number 2	0.7	0.5	0.5
Number 3	0.5	0.4	0.3
Number 4 (near base)	0.6	0.6	0.7

The figures represent seconds. There was no doubt about the comparatively good results with strip 3 and the poor results with strip 1.

The highest point on the contraction curve was reached ordinarily after 15 seconds in all the strips. Complete relaxation followed in from 2 to 5 minutes.

Catalase estimations. In a preceding paper (1) we have shown that the curve of catalase content of muscle from different regions of the small intestine follows closely the curves of rhythmicity and of CO_2 production. This parallelism was so marked as to make us feel that the catalase content of a tissue is a fairly reliable index to its metabolic activity. When dealing with regions like the cecum where one cannot learn much about the rhythmicity, where the latent period is hard to measure and where a number of errors can destroy the value of CO_2 estimations, it is a pleasure to have so simple and apparently so dependable a way of getting at the metabolic gradient.

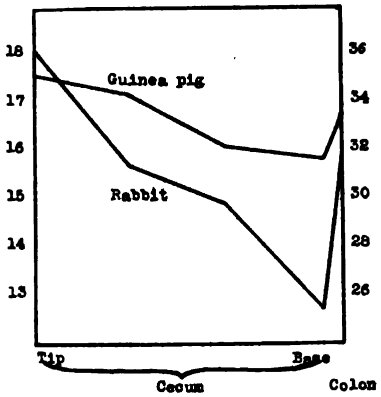

Fig. 2. Ordinates represent average values of catalase in figures of oxygen liberated in fifteen minutes. Those on the left are for the rabbit, those on the right for the guinea-pig. Abscissae represent regions along the cecum and in the colon opposite the cecum.

The method employed was the same as that described in the previous paper (1). The hydrogen peroxid was neutralized before using. All the five estimations were made at the same time under identical conditions of temperature, barometric pressure and shaking. Rabbits and guinea pigs were used. The first four strips were taken from the cecum; no. 5 was from the first portion of the colon opposite the mouth of the cecum. As the guinea pig has no appendix, the first strip was taken next to the tip. In the rabbit the first strip was taken near the base of the appendix. The mucous membrane was scraped off, and the muscle was weighed and ground in a mortar. The following figures represent cubic centimeters of O_2 at atmospheric pressure, liberated in 15 minutes by the catalase in 0.3 gm. of muscle. As the gradation in any one set is the important thing, we have not reduced the data to a common temperature and barometric pressure.

Rabbit

									Average
1. Near tip	16.5	13.5	12.4	23.2	21.2	31.0	15.2	11.8	18.1
2.	15.5	13.3	10.2	19.2	17.0	26.3	14.3	9.9	15.7
3.	15.5	10.4	11.2	14.1	16.5	28.8	12.7	9.5	14.9
4. Near base	11.6	10.3	11.2	8.1	13.0	22.5	14.2	8.7	12.5
5. Colon	15.0	14.1	11.7	14.6	18.5	26.7	16.3	9.4	15.8

Guinea pig

											Average
1. Near tip	36.0	28.7	40.2	23.5	29.4	30.8	37.6	38.6	45.1	43.5	35.3
2.	27.4	23.0	49.2	22.0	26.8	27.7	34.0	34.8	47.4	55.6	34.8
3.	26.1	24.0	37.8	21.0	26.4	27.8	30.8	30.6	43.7	53.7	32.2
4. Near base	26.0	26.2	39.5	21.8	27.5	27.8	33.3	25.5	37.5	50.3	31.5
5. Colon	26.8	25.4	35.7	18.5	26.0	25.6	33.5	31.5	52.5	58.7	33.4

From these results it appears that there is a downward gradient from the tip to the base of the cecum in rabbits and guinea pigs (see fig. 2). From the base the gradient is upwards to the colon. In the guinea pig the difference between the catalase in any two adjacent segments is so near the limit of accuracy of the technic that it is not surprising that, in a number of the animals, we failed to show the usual gradation.

DISCUSSION

We have here to deal with a food reservoir which, in order to retain its contents, must contract but seldom and must not be very responsive to happenings in other parts of the digestive tract. As we should expect, then, we find it lined by muscle of low rhythmicity and low irritability. Judging by its catalase content, its metabolic activity is low. In the rabbit the figures range from 18 at the tip to 12 at the base. In the small intestine of the same animal the figures range from 38.5 in the duodenum to 21.9 in the ileum. It is interesting, in view of the much higher catalase values in the guinea pig, that Elliott and Smith state that the cecum in that animal is much more active than it is in the rabbit.

If the catalase determinations are indices of metabolic activity in the different regions and if, as we believe, peristalsis follows metabolic gradients, then the gradient from the colon to the cecum will explain the filling of the pouch, and the gradient from tip to base will explain the direction of the waves that empty it. In the stomach, small intestine and colon, as far as we have studied them, the gradient of latent period agrees quite closely with the gradients of rhythmicity, catalase content and CO_2 production. There are some exceptions, however. In the intact frog the cardia is much more irritable than the antrum but when strips are excised the cardia is less sensitive than the antrum and its latent period is much longer (8). It seemed pretty clear, from a number of other observations, that the poor reactions obtained with the cardiac strips were due to their great susceptibility to the trauma of excision. The muscle in the antrum, on the other hand, was quite immune to trauma. Similar differences have been observed in all our work with segments of duodenum and ileum. It may easily be, then, that the failure of the latent periods to follow the expected gradient is due to a greater susceptibility to trauma at the tip, where the waves take their origin. It may be, also, that the powerful reactions of strip 3 are due to the presence of a stronger and more efficient muscle at this point, where the large bulk of the contents demands it.

The observations recorded at this time bring added support to the thesis proposed in the previous paper: that the peculiarities of function in the different parts of the digestive tract and the direction of peristalsis in the muscle are to be ascribed to local peculiarities in the muscle (and probably to a much less degree to peculiarities in the nerve net) in the different regions. The low irritability of the cecum may be ascribed partly to the poor development of Auerbach's plexus in this region. Gerlach (9) has shown that the meshes of the nerve net are very large as compared even with those in the ileum. The individual muscle fibers may thus be poorly connected one with the other.

SUMMARY

Excised strips of muscle from the cecum in rabbits and guinea pigs show little tendency to contract rhythmically in oxygenated Locke's solution. The records obtained are different from those traced by segments of small or large intestine. The irritability of the strips is low and the latent periods are long. The low catalase content of the muscle suggests also that its metabolism is sluggish. These peculiarities probably account for the retention of food in this organ for long periods of time.

There is a gradient in the catalase content of the cecal musculature from tip to base. This gradient probably corresponds to a metabolic gradient which determines the direction of peristalsis when waves do appear.

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HISTOLOGICAL STUDY OF FAT CONTAINED IN THE MUCOSA OF THE ALIMENTARY TRACT OF MODERATELY STARVED CATS

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INTRODUCTION

The comparative ease with which fat droplets can be demonstrated in the tissues by means of the various specific stains has greatly facilitated microscopical examination and furnished a stimulus for histological investigation along this line. The present study deals with the presence of these droplets or granules in the mucosa and in the gastric, intestinal and duodenal glands of animals after feeding had been suspended for 24 to 28 hours; i.e., the normal fat constituent, independent of fat absorption.

Many interesting investigations have been made from time to time in an effort to trace the passage of the fat content of the food from the lumen of the gut, through the mucosa and into the vessels of the alimentary wall. With this histological picture the reader is doubtless familiar. So far as I know, however, no previous investigator has interested himself primarily with what may be conveniently termed the normal fat droplets contained in the alimentary tissues, although reference has been made to these or similar bodies by a number of authors.

Eimer ('67), in considering the phenomenon of fat absorption in the small intestine, reported that in some animals one may find individual droplets in the cylindrical cells several days after fat absorption has taken place. He used Schultze's iodine serum for isolating them.

Greene and Skaer ('13) in their histological studies of fat absorption in the mammalian stomach, made the following observations: (1) Traces of fat were found in animals that had been kept without food for 20 to 24 hours, the droplets being located usually at the basal portion of the superficial cells of the mucosa. (2) In puppies and kittens that had not been fed the tissues of the stomach showed the presence of granules, extremely small but possessing the characteristics of fat granules both in appearance and distribution. (3) It was difficult to cause the entire disappearance of fat from the gastric gland-cells by prolonged fasting and occasionally, after moderate to long fasting, the quantity of fat in these cells was even increased. This phenomenon they atrributed to mobilization of body fat. (4) There was a disappearance of fat from the cells during the early stages of fat absorption, which was thought to be due to an increased production of lipase with resulting hydrolization and solution of the fat droplets. In this condition the fat content was not demonstrable by the staining methods used. These investigators made their observations from frozen sections of material that had been fixed in 10 per cent formalin and stained with an alcoholic-alkaline solution of scarlet red.

Mendel and Baumann ('15), in considering the question of fat absorption from the mammalian stomach, resorted to histological methods as a check upon physiological experiments. Cats and dogs were experimented with and pieces selected from the pyloric and fundic regions of the stomach, fixed in Fleming's solution and stained with haematoxylin; or, in a few instances, pieces were fixed in formalin and frozen sections were made and stained with Sudan III. In these experiments there was surgical interference. The authors found few or no fat globules in the mucosa of animals that had been starved for 24 hours. Moreover, in a considerable number of sections practically no more fat was demonstrated in fed animals than in controls, and even when pieces of the stomach had been selected from regions most favorable for the absorption of fat, sections practically devoid of fat were obtained. The distribution was not uniform.

Very interesting findings have been reported by Gay and Southard, ('07) who studied the phenomenon of anaphylaxis in guinea pigs. In seeking pathological lesions they observed a striking picture of fatty change in the walls of the stomach. They examined material fixed with formaldehyde after the Marchi method, supplementing it to a limited extent by examination of fresh tissue in glacial acetic acid and by examination with Scharlach R. In the majority of the cases the animals died within an hour after receiving the second dose of serum. The authors assert that there is no reason to believe that fat is normally present in the gastric mucosa and that the results of their own investigations indicate that in the guinea pig the stomach under normal conditions is free from fat. Moreover the anaphylactic method failed to disclose the presence of fat in the stomach wall. The toxic material, however, showed characteristic fatty changes in various foci while adjacent areas of tissue contained no fat whatever. The controls and anaphylactic stomachs were negative for fat by the Marchi method. The focality of the lesions, the associated congestion and at times the erosion of the surface, were considered to be wholly convincing evidence of the pathological character of the fat shown. Gross hemorrhagic lesions of the stomach were found in a majority of the cases and were always associated with fat in the gastric epithelium and in the endothelium of the neighboring vessels. The authors were inclined to regard the hemorrhages as dependent upon rupture of vessels weakened by fatty change, and the fat of the gastric epithelium as often of independent origin and not necessarily associated with vascular lesions. The fat pictures are illustrated by plates.

EXPERIMENTAL PROCEDURE

From a number of cats experimented with six were chosen from which the observations embodied herein were made. The records kept of these animals are more complete than in my earlier experiments and some of the more obvious technical errors have been avoided. All of the animals were full grown, apparently in good, healthy condition, and the weights of five of them ranged from 3 to 6½ pounds. All were starved for 24 to 28 hours before death. Three of the animals were killed by chloroform, two by a blow on the head and one by gas. Autopsies were performed immediately and the contents of the alimentary tract examined. The lacteals were grossly inspected in two of the cases. As quickly as possible portions from the walls of the stomach, duodenum and lower part of the small intestine were removed from each of the animals and placed in the fixing fluid. The portion of the stomach wall was selected from a segment between 2 and 3 cm. or 2 and 5 cm. above the pylorus. The portion taken from the wall of the duodenum comprised the segment extending from the pylorus to a point 2 or 3 cm. below the pyloric valve. In the later experiments the section taken from the lower part of the small intestine occupied the segment between 32 and 30 cm. above the valve of the colon.

These pieces, taken from the various divisions of the alimentary tract, were fixed in 20 per cent formalin.[1] Four hours were found to be sufficient for fixation but for the sake of convenience this period in some instances was extended. The shortest possible time, however, is preferable (Bullard, '12–'13). After the tissues were taken from the fixing fluid they were washed thoroughly to remove the formalin. Frozen sections were made with the freezing microtome set at 5–15μ. The sections from each division of the digestive tract were divided into two groups. Those of one group were passed through the grades of alcohol (30, 50, 60, 70, 80, 90, 95 per cent) into absolute alcohol or chloroform. Here they were permitted to remain until all the soluble lipoids were removed. These were used as a control for the other specimens. The sections in the other group, which were not treated with absolute alcohol, were passed through 30, 40 and 60 per cent alcohol and then stained with a mixed alcoholic-alkaline solution of Scharlach R and Sudan III.[2]

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[1] The formalin was freshly distilled and diluted with normal salt solution to make up the required strength. (Mann, G.: Physiological histology, 1902, 88).

The staining was done in covered vessels. The granules were found to be satisfactorily colored after the sections had been in the stain for 5 to 10 minutes. Precaution was taken to hurry the washing and hydrating process by running the sections quickly through 50, 60 and 30 per cent alcohol into distilled water, in order to avoid any destaining effect that the alcohols may have upon the fat droplets. It seems best not to allow the sections to remain in the 60 and 50 per cent alcohols longer than 5 to 10 seconds, while in the 30 per cent alcohol a much longer time may be taken without harm. After being removed from the distilled water the sections were lightly counterstained with Erlich's haematoxylin. The mounting was done in either glycerine or sugar.[3] The sections that had been treated with absolute alcohol were first hydrated to 60 per cent alcohol and then stained in a similar manner. Photomicrographs were made from selected sections, a green filter being used. The thickness of the sections made the necessary focusing very difficult.

Gross findings. The autopsies, which were done immediately after death, showed in each case that the stomach and small intestine were empty and collapsed while the large bowel was engorged with fecal matter which at times crumbled when removed. In the duodenal portion of the gut bile-colored mucus could be observed and a trace of perhaps indigestible matter was encountered in the small intestine. There were no evidences of fat absorption when the lacteals were inspected.

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[2] This stain is composed of the folllwing ingredients: Scharlach R, ½ gram, Sudan III, ½ gram, sodium hydroxide, 2 grams, alcohol 70 per cent, 100 cc. (suggested by Dr. H. H. Bullard).

[3] The solution of sugar in which the specimens were mounted was prepared as follows: 70 cc. of distilled water, 15 cc. of a saturated solution of glucose and 5 cc. of glycerine were mixed together. Then 5 cc. of spirits of camphor were added and the mixture filtered.

Microscopical findings. Microscopical examination of sections from the various divisions of the alimentary tract disclosed three types of red objects: (1) Stained fat globules of the submucosa; (2) precipitate; (3) colored fat droplets of the mucosa, and of the gastric, intestinal and duodenal glands.

1. Fat globules of the submucosa: It is not possible to confuse the familiar picture of fat globules normally present in the submucosa with the two other types of stained objects. Apart from their large size and globular form, their location in the submucosa would identify them.

2. Precipitate: Carrying out the directions for staining the fat constituents of the tissue resulted in the production of a precipitate. This might easily have been avoided had the stained sections been washed more thoroughly in 60 per cent alcohol. However, this was deemed unwise because of the risk necessarily involved in subjecting the fat granules to the destaining action of the alcohol. Fortunately, the precipitate possesses certain characteristics that make its differentiation from the fat droplets not only possible but usually quite easy. Three chief points should be borne in mind: (1) The precipitated particles have no definite distribution while the fat granules have; (2) the precipitate may be observed on both surfaces of a section when the microscope is focussed at these two levels, which is not true of the fat granules; (3) it can be distinguished from fat granules by its size and form. Rod- and needle-shaped bodies are sometimes abundant and when once noted are readily distinguishable thereafter. Unfortunately however, these precipitate bodies vary in size and shape, assuming forms that may be confused with the fat granules in the tissues. In these studies such confusion was obviated by always examining two series of sections. It will be recalled that one series was treated with absolute alcohol while the other, from the same divisions of the digestive tract, was not subjected to this treatment. This was done in order that a more definite differentiation between the precipitate and the fat granules might be made. In the sections treated with absolute alcohol it was possible to dissolve out the fat granules and thus destroy the fat picture. Therefore, since all the sections received the same treatment it was possible to examine the two corresponding series—

one containing fat granules and precipitate, the other, only precipitate. This afforded a means of comparison and was always done in instances where there could be any doubt. After a little experience with the precipitate the confusion at first encountered was practically nil. Aside from distinguishing it from the fat droplets, the precipitate was, of course, of no interest.

3. Fat droplets of the mucous lining, and of the gastric, intestinal and duodenal glands: Whereas a large proportion of the precipitated particles are rod-shaped, the normal fat in the mucosa and adjoining glands appears in the form of more or less spherical droplets. These vary considerably in size and are seen collectively only when examined with the low power of the ordinary laboratory microscope. A conception of their size in comparison with that of the cells in which they are found, as well as their abundance in these cells, may be obtained by referring to the photomicrographs, which were made from the various

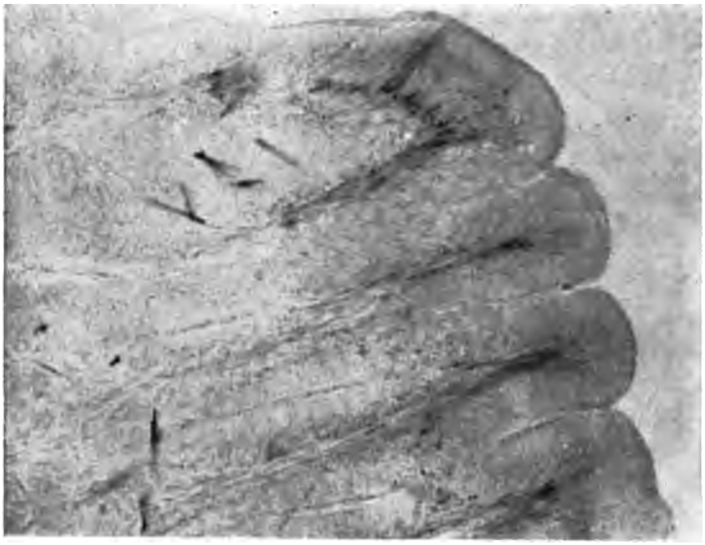

Fig. 1. A photomicrograph of a frozen section of the stomach of a cat, stained with a mixture of Scharlach R and Sudan III. The droplets of stained fat show as dark spots in the bases of the epithelial cells over the surface. Note that they are absent from the cells of the depth of the foveolae. The dark needles are crystals of the stain. The cat was starved for 24 hours and killed with chloroform. × about 293.

divisions of the alimentary tract (see figs. 1 to 6). The amount of fat varied considerably in the different animals. In some unexpectedly large quantities were present while in others the amount was only moderate. In one instance only a trace of fat was found. In general the largest amount was observed in the duodenum or small intestine. By the method employed the fat granules were, of course, stained red and when the microscope was focussed carefully their centers became somewhat yellow. There were no marked differences in the color of the droplets in different divisions of the alimentary tract. The fat granules were extracted from those sections that had been treated with absolute alcohol.

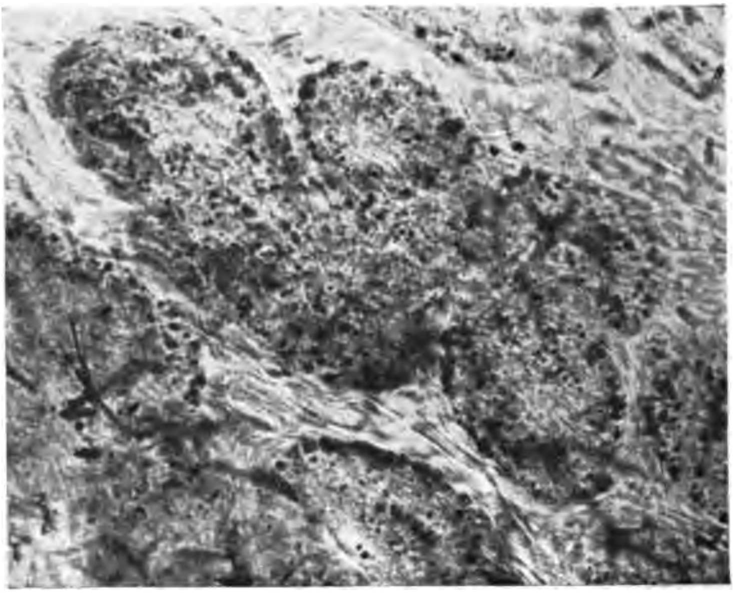

Fig. 2. A photomicrograph of a section of the stomach of a cat showing droplets of fat in the deeper portions or fundus of the gastric glands. The cat was starved about 27 hours and killed by a blow on the head. × about 293.

Fat droplets were present in the gastric mucosa of all the animals, the quantity varying from a relatively large amount to a mere trace. These granules were found in the epithelial cells bordering the mouths of the gastric foveolae (fig. 1). In one of the animals the fat was demonstrated more or less continuously throughout these cells while in others it occupied groups of cells. This focal arrangement recalls the findings of Gay and Southard ('07) referred to above. In the surface cells themselves the granules may appear on both sides of the nuclear zone and one is at a loss at times to determine whether or not they extend into the tunica propria or stroma of the mucosa. They have a tendency to arrange themselves like a string of beads along the long axis of the cells. They do not appear in the outer portion of the cell facing the lumen of the stomach but occupy the basal two-thirds. The nuclear zone itself is comparatively free from them. Similar fat droplets are also present in the fundic or basal region of the gastric glands,

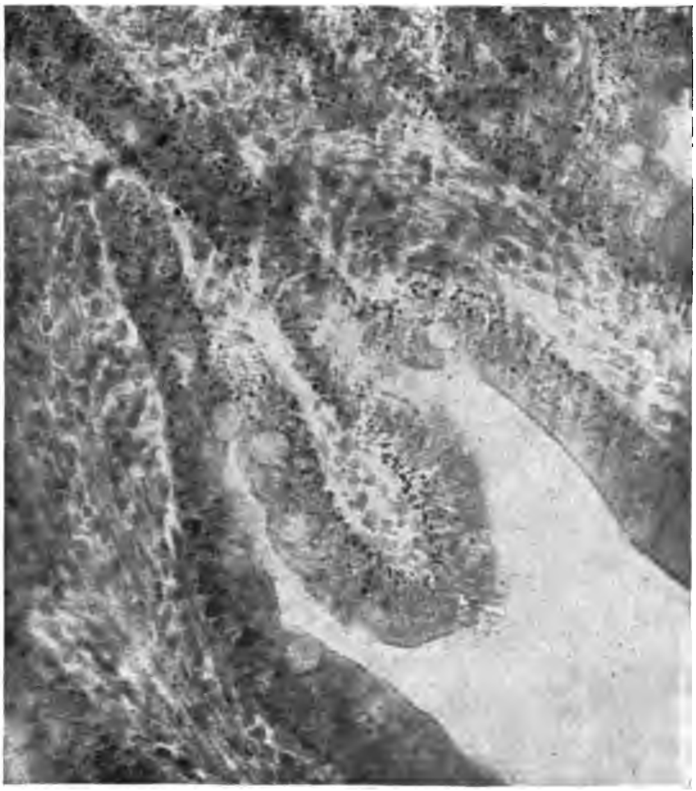

Fig. 3. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in the bases of the villi and the upper portion of the intestinal glands. The cat was starved 28 hours and killed with chloroform. × about 293.

where they vary in quantity from large numbers to a doubtful presence. Figure 2 is a photomicrograph of a section from the stomach in which an unusually large amount of fat was demonstrated. Along the pits, neck and tubules of the gastric glands very few granules were seen and in a number of cases it was very doubtful if any were present. In the sections that showed the granules best they had a tendency to crowd about the base of the cells and appeared to be very similar to those in the border cells, except perhaps that their diameters were greater.

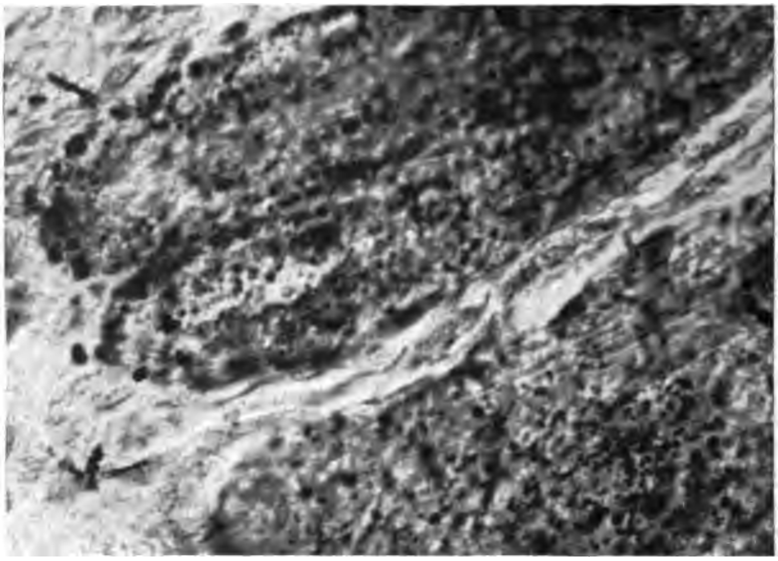

Fig. 4. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in the depths of the intestinal glands. The cat was starved 27 hours and killed by a blow on the head. × about 293.

Obviously, I can agree with Greene and Skaer ('13) as to the presence of these droplets in the stomach mucosa and the gastric glands. The failure of Gay and Southard to demonstrate fat in the gastric mucosa of normal animals may have been due to the staining method employed, or it may be that the gastric mucosa of the guinea pig does not present a fat picture. Certainly there can be no doubt about the occurrence of fat droplets in the gastric mucosa of cats.

In all of the animals fat droplets were demonstrated also in sections from the duodenal wall. As in the gastric mucosa, the quantity varied but the fat picture was at times striking. Usually the droplets were absent from the cells of the mucosa located along the free ends of the villi, while in those located at the base of the villi they were easily demonstrable. Along the intestinal glands the granules were very abundant and extended from the mouths of the glands into their fundic region. Similarly, the granules were found in the duodenal or Brunner's gland. Here the amount varies greatly. In some cases the droplets could not be demonstrated while in others they appeared in great numbers, giving the glands a brilliant red color (fig. 5). At the base of the villi, in chosen sections that demonstrated them well, the fat droplets were located definitely at the basal portion of the cells (fig.

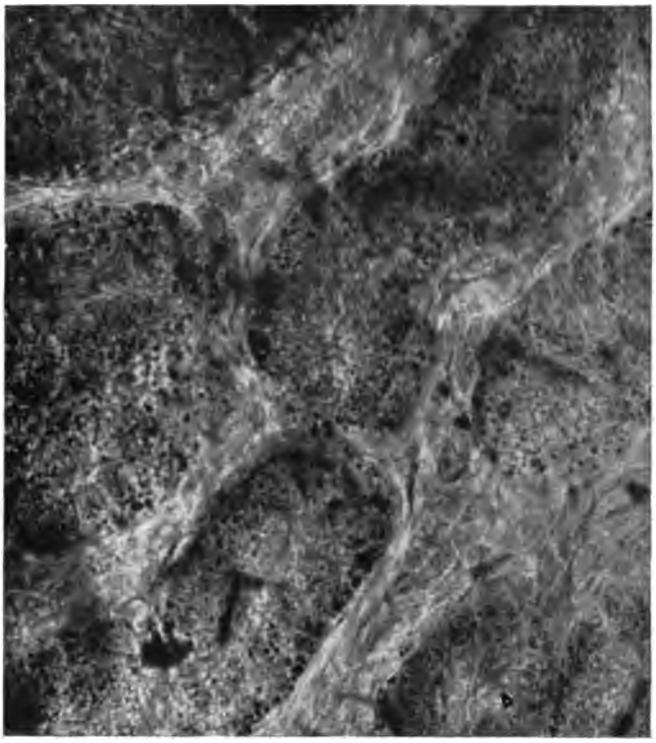

Fig. 5. A photomicrograph of a section of the duodenum of a cat showing droplets of fat in Brunner's glands. The cat was starved 27 hours and killed by a blow on the head. × about 293.

3). It was difficult to locate them accurately in the cells of the intestinal glands, on account of the thickness of the frozen sections and the swollen condition of the glands due to the starvation of the animal (figs. 3 and 4). In the duodenal glands of one of the animals the cells were crowded with these fat droplets, which were larger than the granules found elsewhere in the alimentary tract and any accurate description of their distribution was impossible. The quantity of fat in the cells at the base of the villi as well as in those of the intestinal glands was found to be approximately equal throughout sections from the same animal.

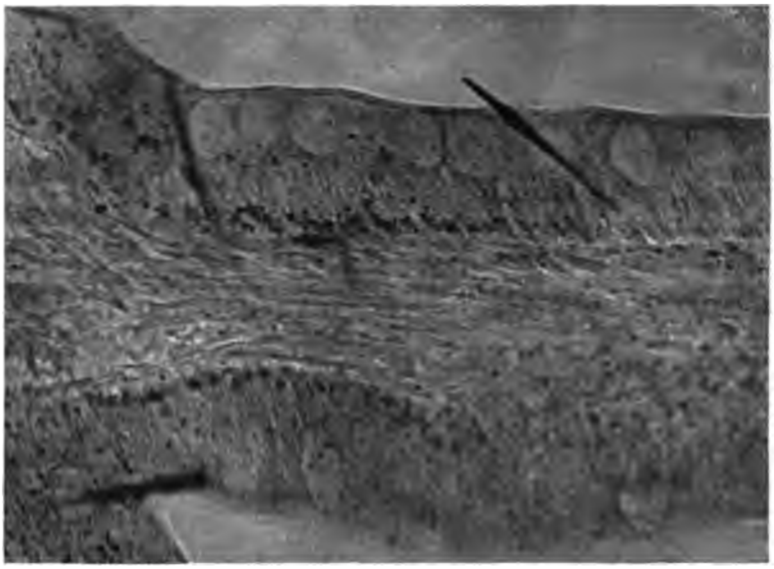

Fig. 6. A photomicrograph of a section of the small intestine of a cat showing droplets of fat in the lower portion of a villus. The cat was starved 28 hours and killed with chloroform. × about 293.

Fat droplets were present in all sections of the small intestine in every one of the animals examined, with the possible exception of one that had been killed by a blow on the head. In another that had met death in a similar manner there were present a large number of the granules. This is another example of the variability of the fat in these animals. In the lower part of the small intestines the granules were abundant along the tubules of the glands. As in the duodenum, the droplets showed a tendency to disappear from the cells of the mucosa as the free ends of the villi were approached, but were very prominent at their base (fig. 6). Here, for the most part, they occupied the basal portion of the cells and toward the free margin were few in number. Due to the thickness of the sections, I was unable to decide definitely whether the droplets were confined solely to the simple columnar epithelial cells or were present in both these and the goblet cells. It is very difficult to describe their distribution in the tubules of the glands. They appear to be more or less evenly distributed but are not uniform in size. In general the picture here is very similar to that described for the duodenal area. In the stroma of the villi, beneath the mucosa, similar droplets may be present. In the stroma between the tubules of the intestinal glands there appeared to be very few.

In seeking an explanation for the presence of the fat droplets several possibilities are to be taken into consideration. The fat picture may be due to any one of the following causes: (1) To fat in process of absorption; (2) to the mobilization of fat; (3) to injury to the cells; (4) to technical errors (a pseudo-fat picture); (5) to the normal fat constituent of the cells.

1. Are we dealing with a fat picture which is the result of fat absorption? There are several counter-indications to this possibility: *a*, These animals were starved from 24 to 28 hours; *b*, there were negative indications of any fat-absorbing phenomenon in progress at the time of autopsy; *c*, it would be difficult to explain by this hypothesis the presence of fat droplets in Brunner's glands.

2. Regarding the mobilization of fat Greene and Skaer, '13, state:

> In the early stages of fasting the labile substances, namely, the carboydrates, are quickly used for the production of energy. A little later the fats of the adipose tissues and other more fixed substances are drawn upon. In this later stage the lipase-producing tissues are doubtless strongly stimulated to increased activity for the accomplishment of the transportation of fats. Lipase was proven by Lovenhart to be a normal content of a large number of tissues of which certain glandular tissues are particularly mentioned by him. To these tissues ought to be added the gastric glands which are lipase producers. If one assumes that an excessive production of lipase takes place in these glands at the time during fasting when the fats are being dissolved from the storage tissues, and are present in a relatively high per cent in the circulating fluids, it follows that there will be an increased synthesis of fat in the lipoid producing tissues themselves.

The presence of fat droplets in the cells may be accounted for thus in some cases but in the present studies, in view of the short period during which the animals were kept without food, it is quite improbable that they had reached the stage of starvation at which fat is being mobilized, as outlined in the above quotation. They were starved 24 to 28 hours, probably not an uncommon occurrence for a cat.

3. A normal amount of fat may be brought to any cell but because of some injury to the cell itself this fat may not be consumed in the normal way and may collect within its borders. Such injuries may be toxic or circulatory. In this instance, however, such an explanation seems quite unsatisfactory. Considering the swiftness with which the animals were killed, it is highly improbable that we are dealing with impaired cellular consumption of fats. Furthermore, it is known that lipoids exist in invisible form within the cells. Injury to the cell may result in disintegrating these lipoids and bringing them into visible evidence. In this way a fat picture may be produced. The question arises, therefore, has any factor been introduced into these experiments that might result in the disintegration of the lipoids in the tissues? This point should be carefully considered, in view of the findings of Gay and Souhard ('07).

For a time it was believed that the fat picture might be accounted for by the assumption that the chloroform used to kill the animals had acted thus on the tissue. For this reason other animals were killed with gas and by blows on the head. Since these methods did not alter the picture to any marked degree, such an explanation would be faulty and questionable.

4. As stated before, in the staining method used in these investigations there resulted a red precipitation. The reason why no attempt was made to avoid this has already been explained. At first glance it may appear that these precipitated particles may have been mistaken for fat droplets. To safeguard against such an error a careful study was made of the precipitate, and moreover all observations were checked by examining fat-free sections with fat-containg sections.

5. It is possible to think of the cells as passing normally through a definite cycle of functional activity, at certain periods of which their lipoid constituents can be demonstrated by histological methods while at others this is not possible. This hypothesis would no doubt account in a more satisfactory manner for the foregoing observations than any of the other possibilities here considered. In those cases in which the fat content was abundantly demonstrated we were dealing, according to this reason, with the period of the cell's activity when the lipoid element was most susceptible for histological demonstration. Again, those cases in which very few or no granules were found represented a period

in the cycle during which the fat or lipoid elements were less susceptible. In this way the variability in the quantity of the fat in the different animals may be explained. Moreover, the presence of fat in such structures as Brunner's glands would in no way conflict with this hypothesis, and the observations of Greene and Skaer ('13) lend support to this suggestion. In their animals they found that the fat content in the stomach disappeared during the early stages of fat absorption. This observation would strongly indicate that these cells may actually possess such a cycle of activity.

SUMMARY

In this paper there has been outlined a histological method by which the fat content of the mucosa and adjoining glands may be satisfactorily demonstrated in moderately starved animals. The appearance of the fat content of these tissues—namely, the superficial epithelium of the stomach and gastric, duodenal and intestinal glands—has been described, due consideration being given to the size, shape, location and quantity of the droplets.

Of various hypotheses the following one has seemed to offer the most satisfactory explanation for the observations noted herein; i.e., that normally there is fat in the epithelial cells of the stomach and intestine which is not associated with the phenomenon of fat absorption; that this normal fat varies, however, with some definite cycle of functional activity of the cells themselves; that at certain periods the lipoids are in such a condition that they can be demonstrated by histological methods while at others they are not demonstrable. The normal presence of these lipoids must be taken into account in estimating experimental results.

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EXTIRPATION OF THE DUODENUM

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The effect of extirpation of the duodenum in the experimental animal has been in dispute since the early work of Minkowski and Pflüger. In 1889 von Mehring and Minkowski (1) demonstrated that complete removal of the pancreas in the dog produced a fatal diabetes. Pflüger (2) found that in the frog diabetes followed total removal of the pancreas but that also a more severe diabetes followed removal of the duodenum or section of the peritoneum between the pancreas and the duodenum. A great deal of experimental work was done on this duodenal-diabetes hypothesis before it was finally discarded.

Ehrmann (3) found that complete removal of the duodenum in the dog resulted in death in several days to a week, with acute pancreatitis and fat necrosis. René Lauwens (4) was able to keep a dog fourteen days after total extirpation of the duodenum. Rosenberg (5) reported five cases of duodenal extirpation in the dog, one of the dogs being still alive twenty-three days after the operation. Minkowski (6) kept a dog several weeks after removal of the duodenum and found only a temporary glycosuria. Bickel (7) also reported a dog living four and one-half weeks after removal of the duodenum. Pflüger (8) criticised the work of these men, claiming that in their experiments not all of the duodenum was removed. S. A. Matthews (9) found that extirpation of the first six inches of the duodenum was invariably fatal. The dogs displayed no serious symptoms for twenty-four to thirty-six hours but very shortly thereafter died. If a piece of the duodenal mucosa was transplanted into the intestine lower down and the remaining duodenum removed, the dogs lived. Death always followed subsequent removal of the transplant. Thus Matthews claimed that the duodenum is as necessary for life as the adrenals or parathyroids, probably through some hormone function other than that concerned with the elaboration of secretin. A. P. Matthews (10) states that if experiments are made so that the duodenal juice (succus entericus) is drained to the exterior through a fistula, the animals die with apparently the symptoms of complete extirpation of the duodenum. He suggests that death may be due to the rapid excretion of some necessary substance through the duodenum to the exterior, or to the loss of some substance normally elaborated by the duodenum which is necessary to the function of the intestine lower down. From work on experimental intestinal obstruction Draper (11) has come to the conclusion that under certain pathological conditions (obstruction) the cells of the duodenal mucosa become perverted and secrete into the blood stream a powerful toxin. Whipple (12) and his collaborators, working on the same problem, have come to somewhat similar conclusions. Under conditions of obstruction the mucosa of the duodenum and possibly jejunum secretes a toxin of a proteose nature both into the lumen of the intestine and into the general circulation. The experimental results published by Draper and Whipple have led clinical men to attribute certain disorders in their patients to some disturbance in this function of the duodenum. Kanavel (13) suggests that death, in rupture of the duodenum, is due not to the ensuing peritonitis but to the absorption of some toxin from the duodenal mucous membrane. Bloodgood (14) thinks that death in some cases of duodenal dilatation likewise may be due to some disturbance of this internal secreting mechanism. He suggests the term "physiologic death."

In their experiments Minkowski, Pflüger and Bickel were intent mainly on severing all nervous connections between the pancreas and duodenum. Apparently they did not have in mind a specific internal secreting function of the duodenum and so may not have taken special care to remove all of the duodenal mucosa. According to S. A. Matthews, a small amount, a patch of mucosa 1.5 x 3 cm., suffices for life. Like Pflüger, Matthews holds that previous workers, reporting successful results, did not remove all of the duodenum.

The question of a specific internal secreting function of the duodenum, aside from the mechanism concerned with the elaboration of secretin, of such a nature as indicated by the work of Matthews, Draper and Whipple, is of great practical importance as well as biologic interest. We became interested in the question during the course of some work on the nature of the toxemia in intestinal obstruction and experiments of the following nature were performed.

Extirpation of varying lengths of the intestine. Removal of varying lengths of the jejunum and ileum produced no other effect than some nutritional disturbance similar to that described by Underhill (15) in experiments on dogs in which the small intestine was short circuited (functionally resected). The mucosa of the combined jejunum and ileum does not secrete or manufacture a necessary substance and animals can survive for months after the removal of the small intestine, the duodenum and colon remaining intact. All of the effects can be accounted for simply as due to the loss of an important digestive and absorptive organ.

Extirpation of the duodenum. Removal of the duodenum presents greater surgical difficulties. Because of the extreme vascularity of the duodenum, the intimate relation to the liver, stomach and pancreas many animals die from shock, internal hemorrhage or acute pancreatitis. There is always a profound disturbance of the functions of the liver, stomach and pancreas. The duodenum was removed from sixteen dogs and their behavior carefully observed. In each case the pyloric part of the stomach, the entire duodenum and the first part of the jejunum were removed. This of course necessitated a dissection of the ligament binding the lower duodenum and upper jejunum in order to permit the reëstablishment of intestinal continuity. In eleven of the dogs the operation was performed in two stages. At the first operation the pylorus was divided, both ends closed and an anterior gastro-enterostomy performed with the middle jejunum. After recovery from the first operation, a second was done and the duodenum removed as far as the gastro-enterostomy. The bile and pancreatic ducts were tied and the gall bladder drained, in some cases into the jejunum, in others to the exterior. Most of these dogs died in two or three days but one lived twelve days. At autopsy there was usually a general abdominal fat necrosis and it is our opinion that this, combined with the unavoidable injury to the pancreas in separating it from the duodenum, is the cause of death in these cases. The remainder of the dogs were operated in the following manner. The common bile duct was ligated and cut, the pylorus divided, the pancreas opposite the pylorus ligated and divided with a cautery and the entire duodenum with the adherent pancreas as well as the upper jejunum removed. Enough of the pancreas was left to prevent the onset of diabetes. As evidenced by the post-operative course of these animals and by subsequent post mortem examination, there was less danger of producing pancreatitis with this operation than when the pancreas was separated from the duodenum. The middle jejunum was sutured to the divided pylorus and the gall bladder drained. The operation was completed at one stage and this proved to be a great advantage over the two-stage operation because of the extensive adhesions in the latter case. There was less gastric disturbance when the jejunum was sutured directly to the cut pylorus than when a gastro-enterostomy was done. All of the dogs were given glucose dissolved in Ringer's solution hypodermically daily for the first week. Most of these animals recovered from the immediate effects of the operation, displayed no untoward symptoms for five or six days but very shortly thereafter died. One of the dogs lived three weeks and one three months after the complete extirpation of the duodenum. Confirming the observations of Minkowski, these dogs showed only a temporary post-operative glycosuria and soon became sugar-free, remaining so till death.

The dog that survived three months showed, naturally, a very marked nutritional disturbance and it was only by careful feeding and attention that it was kept living for that period. There was a constant loss of body weight in spite of a liberal diet of lean meat and sugar. That there was an incomplete digestion of the food fed was evident from the almost constant appearance of undigested meat in the feces. It was soon apparent that the animal was starving in spite of efforts to maintain nutrition. Apparently the gastric juice alone, in the absence of bile, pancreatic and duodenal juice, does not suffice for the utilization of proteins. It is probable that the animal could have been preserved indefinitely in nitrogen equilibrium if a sufficient amount of protein in the form of peptones or amino acids had been supplied, along with sufficient carbohydrates for energy purposes. The symptoms produced in this dog by the complete extirpation of the duodenum are in all probability due simply to the disturbance in digestion. The duodenum or duodenal mucosa is not itself essential for life and is certainly not comparable to the adrenals or parathyroids in this respect. These experiments also establish the fact that the duodenum does not supply a substance which is necessary to the function of the intestine lower down. Intestinal motility was normal, or if anything exaggerated, after the removal of the duodenum.

The question of the toxicity of normal intestinal juice. Some workers (Draper) have concluded on the basis of animal experiments that the secretion of the duodenum and upper jejunum is toxic but that it is normally neutralized by the secretions of the intestinal mucosa lower down. One of us during the course of some experiments on the toxemia of intestinal obstruction found that dogs could survive open isolated loops of the dodenum or jejunum in which the respective secretions are poured drectly into the abdominal cavity and absorbed. These dogs displayed no toxic symptoms. In a recent communication Davis and Stone have verified our contention that the succus entericus is not toxic when secreted. They found that the fresh secretion produced no untoward symptoms when injected intravenously in dogs.

The question of the toxicity of intestinal juice under pathological conditions. Whipple and his coworkers have concluded that under certain pathological conditions (obstruction, closed intestinal loops) the mucosa of the duodenum and jejunum assumes a perverted function and secretes a toxic proteose both into the lumen of the intestine and into the blood stream. When such pathological conditions are produced in the experimental animal there is no doubt that poisons accumulate in the obstructed intestine or in closed intestinal loops and that the dog shows symptoms of marked toxemia. In the experiments reported it seemed entirely possible that the toxic substances could be the products of bacterial activity. We know that exceedingly toxic substances such as the amines are produced in the intestine by the action of bacteria on certain amino acids. It seemed that the presence of bacteria should be definitely excluded before any conclusions were made regarding the toxicity of the secretion as it is formed by the duodenal cell or as it is found in the lumen of the intestine. When such precautions were taken—isolated intestinal loops rendered sterile by prolonged drainage into the abdominal cavity—it was found that many procedures, formation of closed loop, complete occlusion of the blood supply with resulting autolysis of the loop, which invariably caused severe toxemia and death when bacteria were not excluded, did not produce any noticeable symptoms.

Does the duodenum excrete some necessary substance in the duodenal juice? According to A. P. Matthews, if the duodenal juice is drained to the exterior through a fistula, death always results with apparently the same symptoms displayed by animals following a complete extirpation of the duodenum. The duodenum may excrete some necessary substance in the duodenal juice which is normally reabsorbed by the intestine lower down. A number of experiments were done to determine this point. If a fistula of the duodenum below the entrance of the bile and pancreatic ducts (fig. 1) be made the animals usually die within three days.

If however the operation be done as in figure 2, so that there is no blind stump of the first part of the duodenum, some of the animals live indefinitely and show no untoward symptoms whatever. The secretions of the duodenal mucosa below the entrance of the bile and pancreatic ducts do not contain any substance necessary to the organism.

If the secretions of the entire duodenum be drained by means of an abdominal fistula, the situation is similar to that following complete extirpation. The injury to the liver, pancreas and stomach as a result of the operation, together with the loss of three important digestive juices, presents such an abnormal situation that one need not postulate the loss of some necessary substance to explain the death that usually ensues. A number of dogs were operated as in figure 3. Most of the animals died within three days but two lived six days and one ten days. Dr. James J. Moorhead of Chicago was able to keep a dog twelve days after an operation similar to that described above. In addition to the disturbance in digestion produced by the deprivation of bile, pancreatic and duodenal juice, a slight occlusion of the fistulous opening in the skin produces a dilatation of the entire duodenum, which in itself is a

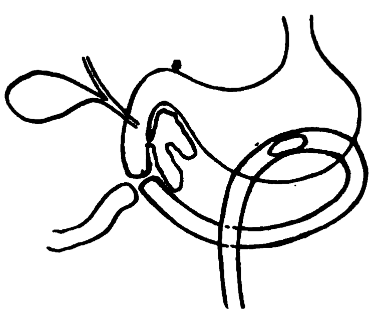

Fig. 1

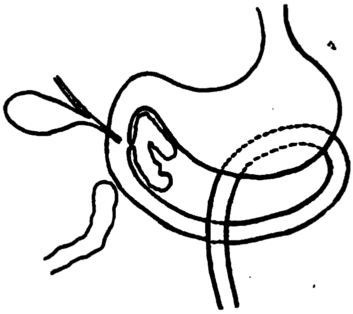

Fig. 2

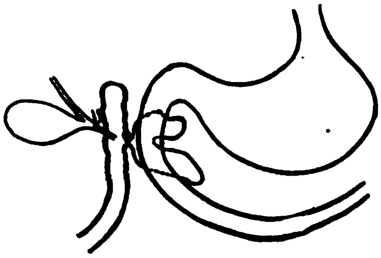

Fig. 3

serious condition. For this reason this operation was not as successful as complete extirpation of the duodenum. There is no evidence, however, that the duodenal juice contains anything vitally necessary to the organism.

CONCLUSIONS

1. Animals can survive indefinitely a complete extirpation of the combined jejunum and ileum.
2. A dog was kept three months after a complete removal of the pyloric part of the stomach, the entire duodenum and the upper jejunum. The mucosa of this region of the digestive tract is not comparable to the adrenals or parathyroids in function.
3. The normal secretions of the duodenum and jejunum are not toxic.
4. When bacteria are excluded from the lumen of the intestine, various pathological changes even to complete occlusion of the blood supply to an isolated piece of intestine with resulting autolysis and reabsorption, can take place without the elaboration of sufficient toxic substances in the cells themselves or in their secretions to kill the animal.
5. The duodenum does not excrete in the duodenal juice any substance necesssary for life or for the function of the intestine lower down.

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THE VENO-PRESSOR MECHANISM

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This paper is the outcome of a suggestion by the Committee on Physiology of the National Research Council that the author investigate the question of a veno-pressor mechanism. It was believed that the derangement of such a mechanism might be a contributing factor in the stasis of blood which, in most instances at least, characterizes the condition of shock.

The relationship of the facts observed to shock has not been studied. It is not improbable that there is a relationship but it is not one easy of exact demonstration. The facts, however, are of interest in themselves and their bearing upon the larger problem, if there is such bearing, may be left to the future.

EXPERIMENTAL

Dogs were used and the experimental results are limited to observations on the sigmoidal area of the large intestine, approximately 8 cm. in length. The part receives its blood supply chiefly through a large branch of the inferior mesenteric artery and is drained by a good sized mesenteric vein. It is innervated by a nerve trunk originating in the inferior mesenteric ganglion which runs to the gut closely adherent to the artery. The technical procedure followed was to lay double mass ligatures about the gut and to cut between them above and below this area, to sever the intervening mesentery and to suspend the preparation by the ligatures about either end so that it hung free of pressure disturbances from the neighboring coils of intestine. The vein and artery were then cannulated and the blood washed out of all the vessels by arterial perfusion with warm Ringer's solution. In the earlier experiments this washing was followed by perfusion with Ringer's solution containing a suspension intended to produce capillary block. In the later experiments this step was omitted because of the lack of assurance that the block was in the capillaries and not in the arterioles at a point such that contraction of these vessels might contribute to the rise of pressure in the vein. A further reason for its discontinuance was that the rise of pressure in the vein occurred whether the blocking suspension was introduced, whether the artery was simply clamped or whether the artery was left open. The final practice adopted then was to leave the artery open and it was assumed that if arterial contraction did occur it would tend to pass the contained fluid backward through the open artery rather than into the vein. The necessity for such an assumption is unfortunate; nevertheless, since the pressure on the arterial side was, by the procedure, at zero or even below zero (the open artery hanging down from the suspended gut) while the pressure on the venous side was 6.0 to 10.0 cm. of water, the likelihood of arterial constriction contributing to the rise of vein pressure is small. It is possible that arterial constriction by a peristaltic wave may sweep fluid onward into the capillaries and veins even when the arterial pressure is extremely low. But such activity is not demonstrable by the usual methods of studying vasomotor activity. In other words, vasoconstriction as usually produced is accompanied only by a fall in venous pressure. Under the experimental conditions, then, the observed rise of venous pressure whether due to constriction of arterioles, capillaries or veins would seem of necessity to be associated with a veno-pressor mechanism since the effect is to produce an active rise of venous pressure.

Attempt was made to produce a capillary block by perfusion through the vein instead of through the artery. This proved to be impossible, as has been noted by Mall, because of the valves. After the artery was relieved of the excess of perfusion pressure there was apparently little or no tendency for the fluid on the venous side to leak back, presumably because of valves, as indicated by the stability of the venous pressure.

After the blood was completely washed out of the vessels the vein was connected with a water manometer which served to record changes in venous tone. In most of the experiments in addition to the vein manometer a second manometer of the same bore was connected with the lumen of the intestine, a cannula having been included in one of the mass ligatures for this purpose, so as to follow concomitant changes of pressure due to contraction of the intestinal musculature.

The pumping action of the intestinal villi observed by Hambleton (1) as a possible factor in the venous blood pressure changes noted in these experiments has not been considered. It is conceivable that such a factor is involved but no means are at present available for its investigation.

THE PERIPHERAL MECHANISM

With the preparation as above described, it is an easy matter to demonstrate the peripheral veno-pressor mechanism. Section and peripheral stimulation of the nerve trunk running from the inferior mesenteric ganglion to the part under investigation almost invariably gives a distinct rise of pressure in the vein as read on the water manometer. This result is as easily obtained if the preparation is entirely removed from the body and the reaction may be obtained for an astonishingly long time. In an experiment directed particularly to this point a good reaction was shown upon faradic stimulation of the nerve an hour and fifteen minutes after the circulation was isolated.

In a series of experiments notes of thirty-one observations were preserved showing a rise of vein pressure independent of pressure changes within the lumen of the gut. There is usually a latent period of some seconds after which the rise of pressure sets in. The rise is slow and steady and if the stimulus be of short duration may reach its maximum some time after the irritation has been removed. On the other hand a protracted stimulation results in a sustained elevation of the pressure. By the end of the second minute of such stimulation the preparation shows signs of fatigue and the vein pressure begins to fall. It was not determined whether this fatigue is localized in the nerve fiber, in the vein or in both.

In this series of thirty-one observations the rise of vein pressure in centimeters of water was as follows: 2.0, 2.0, 3.25, 0.75, 1.0, 7.25, 4.5, 4.5, 2.0, 2.5, 1.0, 0.75, 1.25, 0.75, 1.25, 0.75, 1.0, 1.5, 7.0, 5.0, 2.75, 2.25, 0.75, 1.75, 1.5, 1.0, 2.0, 1.75, 0.75, 4.5, 5.5, 1.75 and 1.5 cm. These figures show a minimum rise of 0.75 cm. and a maximum rise of 7.25 cm. Within limits the strength of the stimulus determines the extent of the rise of pressure but the chief factor appeared to be the condition of irritability of the preparation for at times the strongest stimulation fails to elicit any response and the greatest rise is not necessarily associated with the strongest stimulus. After the maximum has been reached the pressure falls back slowly so that the curve resembles a curve of the contraction of smooth muscle.

THE CENTRAL MECHANISM

The part played by the central portion of the veno-pressor mechanism is much less easy of demonstration. This was studied by leaving the nervous connection to the part intact. Time and again when the peripheral mechanism gave a ready response to direct nerve stimulation no response was elicited through the central mechanism so that the conviction was firmly established that the nerve centers controlling the regulation of venous tone are prone to lose their functional power under the abnormal conditions established by the experimental procedure. The operative exposure of the abdominal viscera and manipulations incident to the procedure subjected the anesthetized animal to extreme sensory stimulation adequate, it may be assumed, to start the train of events which ultimately leads to the condition of shock.

Under the most favorable experimental conditions a reflex rise of venous pressure was demonstrable but it could not be demonstrated repeatedly and was never demonstrated late in an experiment. But so far as the actual demonstration of the existence of a central veno-motor mechanism goes and aside from its possible significance in shock, we are concerned wth the positive evidence of central regulation.

The line of attack in these experiments was twofold: to study the effect upon the venous pressure of nerve section or of section of the spinal cord (pithing) and of central stimulation produced by sensory nerve irritation or by asphyxia.

Fall of venous pressure incident to nerve section. The simplest method of determining the presence of central venous tone was to cut the nerve and note the consequent fall in venous pressure. This procedure was instituted only when the vein manometer gave a constant reading. In seven experiments the fall in venous pressure was noted together with the time required for the manometer to again give a constant reading. The results were as follows: 2 minutes, 1.0 cm.; 4 minutes, 0.5 cm.; ½ minute, 0.5 cm.; 19 minutes, 4.0 cm.; 9 minutes, 4.0 cm.; ½ minute, 0.5 cm. and 2 minutes, 0.5 cm.

Fall of venous pressure incident to pithing. The animal was pithed by passing a small scalpel through the tissues at the base of the skull and transecting the cord at the foramen magnum. The effect of this procedure was usually to cause a transitory rise of venous pressure followed by a slow fall, the latter being like that observed upon nerve section. The experiments on cord transection, as above described, three in number, served to locate the central veno-pressor mechanism in the medulla. The results were as follows:

1. After 15 minutes pressure fell 4.0 cm.
2. After 1 minute pressure rose 1.5 cm.
   After 10 minutes pressure fell 3.25 cm. below original level.
3. After ½ minute pressure rose 1.0 cm.
   After 9 minutes pressure fell 0.5 cm. below original level.

Not much emphasis is to be laid upon these results or upon the results of nerve section. They indicate an influence of a medullary center upon the venous tone but the evidence is not so striking as might be wished because of the slowness with which the change is brought about. Possibly there is little central tone under the experimental conditions or possibly the load-tension on the vascular muscle was insufficient to cause prompt relaxation. In any case the results are much less convincing than those which exhibit a rise in pressure upon sensory stimulation.

Rise of venous pressure incident to faradic stimulus of saphenous nerve. The saphenous was chosen as a convenient sensory nerve for the study of reflex effects upon the venous blood pressure and all the results reported were obtained by its stimulation. In eight experiments twenty-two observations were made which gave a rise of venous pressure. In these the rise of vein pressure in centimeters of water was as follows: 1.0, 0.5, 0.5, 0.75, 0.5, 2.0, 1.25, 1.5, 1.5, 2.0, 3.5, 2.0, 0.5, 1.5, 1.5, 1.0, 0.5, 1.0, 0.5, 1.0, 0.5 and 0.75 cm. These figures show a minimum rise of 0.5 cm. and a maximum rise of 3.5 cm. In the case of the peripheral mechanism the chief factor controlling the response appeared to be the irritability of the preparation. So here, in the case of the central mechanism, the irritability of the veno-presso center rather than the strength of stimulation was the controlling factor.

It will be noted that the rise of pressure produced reflexly by sensory nerve stimulation is generally less than the rise produced by direct stimulation of the veno-motor nerve fibers themselves—the maximum rise for the latter being 7.5 cm. as compared with 3.5 cm. for the former. This was not unexpected since the mediation of the central mechanism would predicate a lesser response. It should perhaps be stated that these results were wholly independent of pressure changes within the lumen of the gut and of pressure from neighboring viscera.

Rise of venous pressure incident to asphyxiation. Occlusion of the trachea proved to be the most satisfactory way of producing a rise of pressure by the activation of the veno-pressor center. Not infrequently when sensory nerve stimulation was without effect a considerable rise in vein pressure was produced by asphyxiation and the inference is made that asphyxia is the more potent stimulus. In eight experiments eleven observations were made. In these the rise of venous pressure in centimeters of water was as follows: 5.0, 2.75, 1.25, 2.5, 1.5, 2.25, 4.5, 1.5, 4.0, 3.25 and 2.0 cm. The minimum rise was 1.25 cm. and the maximum rise was 4.5 cm. In several experiments a preliminary slight fall in pressure was noted which must have been produced by a transitory loss in central tone. This fall, however, shortly gave place to a rise in pressure which sometimes progressed beyond the period of occlusion of the trachea. In two experiments the manometer readings were strikingly confirmed by observation of the intact loops of intestine lying near the preparation. In the beginning the larger veins particularly along the mesenteric border were distended and conspicuous. As asphyxia progressed these veins decreased in size and became relatively inconspicuous. Along with this change in the appearance of the veins the intestine became blanched. This change in the appearance of the veins can not be explained by an asphyxial constriction of the arterioles because this would not empty the veins and in the present case the writhing movements which accompany asphyxia were unusually slight and did not appear until after the change described in the veins had developed. A similar change in the veins in the part connected with the manometer was not apparent because these veins had been washed free of blood and were therefore relatively inconspicuous. The veins which showed this reaction were, of course, filled with asphyxial blood and it is possible to explain the constriction on Henderson's hypothesis (2) of the chemical veno-constrictor effect of carbon dioxide. The writer is not, however, impressed by this hypothesis especially because of repeated confirmation of Bayliss' earlier observation that carbon dioxide relaxes vascular muscle (3). In view of the results reported in this paper it seems very probable that the constriction noted in the veins was associated with the central (nervous) effect of the asphyxia.

In the asphyxia experiments as well as in the experiments dealing with saphenous stimulation care was necessary lest the excessive respiratory movements result in compression of the preparation in such a way as to raise passively the vein pressure. To obtain assurance that such a passive factor was not in force unknown to the observer an experiment was performed in which after obtaining an asphyxial rise of vein pressure in the usual manner the nerve to the part was cut and asphyxia again instituted. With the nerve cut an extreme asphyxia was entirely without effect upon the vein pressure.

INTESTINAL MECHANISM

Under this heading are considered observations in which active changes in the intestinal musculature obviously contributed or at least occurred coincidently with considerable alterations of the vein pressure. The data at hand are quite limited and no effort was made to study the condition carefully. In the cases in which it occurred the vagi were intact and ether alone was used for anesthesia. It would seem that under certain conditions intestinal movements may materially influence the movement of blood in the portal system as suggested by Mall. At times the pressure within the intestinal loop remains unchanged during a rise of vein pressure, at times it shows a decided fall and at times again it may show a decided rise. To give an example which shows the coincident rise of both loop and vein pressures it was found that with vagi intact and with ether alone as anesthesia, stimulation of the saphenous nerve and asphyxia gave the following pressure changes:

	VEIN PRESSURE ROSE	GUT PRESSURE ROSE
	cm.	*cm.*
First saphenous stimulation	7.0	24.0
Second saphenous stimulation	3.5	8.5
Third saphenous stimulation	1.5	17.0
Occlusion of trachea	5.25	25.0

It is clear that in a case such as this the rise of vein pressure is probably in large part at least passive. This function of intestinal movement may well be of significance under normal conditions although it is not to be regarded as essential to the veno-pressor mechanism.

The reader will appreciate that the results given above represent an extension of the observations made by Mall in 1896 (4). Mall showed that splanchnic stimulation causes constriction of the portal vein and its tributaries. Recently Burton-Opitz (5) has confirmed these observations by another method. Mall, then, demonstrated the existence of a peripheral veno-pressor mechanism and it was a natural inference that such a mechanism should be under central and therefore reflex control.

Recent contributions to the shock problem (6) emphasize a chemical factor as determining the failure of the circulation. According to this view some toxic metabolic product dilates the capillaries and venules producing a peripheral stasis of blood. That a nervous control is not to be excluded is indicated by the experiments of Cotton, Slade and Lewis (7) which show that these small vessels respond to stimulation as was shown by Stricker in 1865 (8). It is possible that both nervous and chemical factors may contribute to vascular relaxation in the capillary region in shock. If so, we should expect the more promptly acting nervous mechanism to instigate the changes which are subsequently accentuated by the processes due to chemical action.

SUMMARY

1. A preparation of the large intestine in the dog is described, on which a rise of venous pressure may be demonstrated independently of arterial and other factors.

2. The rise of venous pressure may be obtained by *a*, stimulation of the nerve to the part (peripheral mechanism); *b*, stimulation of a sensory nerve (central reflex mechanism); and *c*, central stimulation by asphyxia.

3. Attention is called to the fact that activity of the intestinal muscle may contribute to the movement and pressure of blood in the portal venous system.

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